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# THE INDIAN JOURNAL

## OF

# GENETICS & PLANT BREEDING

CONTENTS



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# The Indian Society of Genetics & Plant Breeding

The Society was founded in January 1941 with the following objects:

1. To advance the cause of Genetics and Plant Breeding in India and to encourage and promote study and research in these subjects.
2. To disseminate knowledge of Genetics and Plant Breeding.
3. To provide facilities for association and conference among students of heredity and for the encouragement of close relationship between workers in Genetics and Plant Breeding and those in the related sciences.



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# The Indian Journal of Genetics & Plant Breeding

Edited
by
B. P. Pal

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Dr. B. P. Pal

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OF
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## RAFI AHMED KIDWAI MEMORIAL PRIZE

To commemorate the memory of the late Shri Rafi Ahmed Kidwai and to recognise suitably any outstanding research work done by scientists in the field of agriculture and animal husbandry, the Indian Council of Agricultural Research instituted in 1958 11 prizes known as "The Rafi Ahmed Kidwai Memorial Prizes for Agricultural Research". The first Rafi Ahmed Kidwai Prize for significant research in the field of Agricultural Botany was awarded in September, 1960, to Dr. B. P. Pal, Director, Indian Agricultural Research Institute, New Delhi, for the famous Pusa wheat varieties bred by him. Dr. B. P. Pal is a founder-member of the Indian Society of Genetics and Plant Breeding and has also been the Editor of the Indian Journal of Genetics and Plant Breeding ever since its inception in 1941. The Executive Council and members of the Indian Society of Genetics and Plant Breeding wish to convey through the columns of the Society's Journal their warm felicitations to Dr. Pal on this award and to wish him many more years of distinguished service in the cause of Indian agriculture.

Dr. B. P. Pal had his early education in Burma where he took the M.Sc. degree in Botany with First class Honours from Rangoon University in 1929. Subsequently, he went to Downing College at Cambridge University where he did his Ph.D. in Plant Breeding and Genetics under the renowned Professors, the late Sir Rowland Biffen and Sir Frank Engledow. His work at Cambridge clearly established the possibility of exploiting the phenomenon of hybrid vigour for the improvement of self-pollinated cereals. On his return to India, he joined the Indian (then Imperial) Agricultural Research Institute at Pusa as Second Economic Botanist. In 1937, he became the Imperial Economic Botanist (this post was later designated as Head of the Division of Botany). He was appointed as Director of the Indian Agricultural Research Institute in 1950.

Dr. Pal is well-known internationally for the work he has done in the field of wheat breeding and genetics. The variety N.P. 809 bred by him and released by the Indian Agricultural Research Institute in 1954 represents a landmark in the history of wheat improvement work in that it is the first variety to possess resistance to all the three wheat rusts. Shri K. M. Munshi, then Governor of Uttar Pradesh, (and formerly Union Minister of Food and Agriculture) during a talk delivered on the occasion of the Golden Jubilee of the Indian Agricultural Research Institute in April, 1955, estimated that the improved rust-resistant wheat varieties bred by Dr. Pal are responsible for an increased income to the farmers worth Rs. 25 to 30 crores annually. In recognition of the outstanding research done by Dr. Pal in the fields of genetics and plant breeding, the Genetics Society of Japan elected him as an Honorary Member in 1956. He was awarded "Padma Shri" by the President of India in January, 1958.

A summary of some of the important scientific contributions made by Dr. Pal during the last 30 years is given below.

*Breeding new varieties of wheat:*

Realising that the improvement of wheat yields in India would depend largely on our ability to control the severe losses caused to the crop by rust infection, Dr. Pal initiated a breeding programme for evolving rust-resistant wheat varieties in 1934. In collaboration with the late Dr. K. C. Mehta and others, he bred several varieties individually resistant to the different rusts. He combined the genes for rust resistance with those controlling good grain quality and high yield and the varieties New Pusa 710, N.P. 718, N.P. 761 and N.P. 770 were thus evolved. These varieties are widely grown in many States of India. Later, he developed the highly rust-resistant varieties N.P. 797, N.P. 798 and N.P. 799 which are in addition high yielding, early in maturity and resistant to loose smut. During the severe rust epidemics in Bihar and West Bengal in 1956-57, these varieties were healthy and gave good yields while the local varieties were completely destroyed. Dr. Pal is the chief architect of the "Coordinated Wheat Rust Control Scheme", a project under which varieties resistant to the different rusts and suitable for cultivation in all the wheat growing States are being bred. To organise the breeding work carried out under this project on sound lines, he initiated work on several aspects like the preparation of a National Register of Genetic Stocks of Wheat, the utilization of exotic varieties either as breeding material or for direct cultivation and the study of the milling, baking and chapati-making qualities of Indian Wheat varieties. He also established a germplasm bank of wheat and initiated work to take advantage of the genome approach to wheat breeding.

As mentioned earlier, the variety N.P. 809 which is the first wheat variety to possess resistance to all the three rusts, was released in 1954. This variety was the result of many years of planned breeding work carried out by Dr. Pal with the collaboration of plant pathologists. With the setting-up of fertilizer factories within the country and the consequent possibilities for undertaking intensive cultivation, Dr. Pal anticipated a need for varieties which respond well to heavy fertilization. He consequently re-oriented the wheat breeding programme so as to include good response to manuring as one of the criteria for selection. As a result, several new varieties such as N.P. 823, N.P. 824 and N.P. 828 which respond well to the application of heavy doses of fertilizers and at the same time are resistant to rust and loose smut diseases, have been evolved. Another variety, N.P. 825, has given high yields under conditions of drought and may become a boon to the farmers cultivating wheat under rain-fed conditions. To solve the handicap which the lack of precise knowledge concerning the inheritance of characters of agronomic interest causes in breeding work, Dr. Pal intensified the genetic studies in wheat. Several lines of approach were followed. First, by using classical methods the genetics of rust resistance and several morphological characters were studied in many wheat varieties. This knowledge helped in understanding the nature of genetic control of rust reactions more fully. In a paper presented at the International Genetics Symposium held in Japan in 1956, Dr. Pal clarified the genetic situation concerning rust resistance in wheats. He showed that two sets of genes—one conferring rust resistance and another inhibiting the resistance reaction—are present in wheat varieties and that the dominance or recessive nature of rust resistance may vary in different crosses depending upon which set of genes is involved. He, thus, clarified a complex genetic situation arising from the variable dominance relationships found with reference to the same characters in different crosses. A second line of approach initiated by Dr. Pal for the study of the genetics of Indian wheat varieties is monosomic analysis i.e., using plants deficient for individual chromosomes to locate the genes situated on those chromosomes. The chromosomes on which genes conferring resistance to races of black, brown and yellow rusts were, thus, identified in different varieties and experiments are in progress to transfer selectively those chromosomes to susceptible wheat varieties. A comprehensive monograph on wheat prepared by Dr. Pal is currently under publication by the I.C.A.R.

*Identification and classification of varieties of crop plants:*

Soon after starting his work on the breeding of improved varieties of Indian crop plants, Dr. Pal realised the need for a thorough re-classification of the varieties of different crop plants and for the formulation of suitable keys for identifying the different varieties. He himself undertook this work in wheat and potato and published comprehensive papers on the classification and identification of the varieties of these crops. Thus, he brought to an end a chaotic situation which existed in the field of varietal nomenclature in India. Dr. Pal has also taken an active interest in the revision of the International code of Nomenclature of Agricultural and Horticultural Plants. In pursuance of his interest in evolving suitable keys for the identification of wheat varieties, Dr. Pal explored the use of anatomical characteristics in classification and found that the structure of leaf hairs was very useful for this purpose.

*Inter-specific relationships in Triticum:*

An important contribution to the available knowledge concerning the taxonomic relationships among hexaploid *Triticum* species was made by Dr. Pal in 1957, when in collaboration with one of his colleagues, Mr. H. B. Singh and the eminent American geneticist, Dr. Edgar Anderson, he published a paper on the genetics of *Triticum vavilovi*. *T. vavilovi* is one of the lesser known hexaploid wheats. By studying the progenies of crosses between *T. vavilovi* and the cultivated species, *T. vulgare* and *T. sphaerococcum*, it was shown that *T. vavilovi* is more closely related to *T. vulgare* than might be inferred from a superficial study. He has also suggested that *T. vavilovi* and the other cultivated hexaploid *Triticum* species should all be considered as subspecies of *T. aestivum*.

*Utilization of Hybrid Vigour in Crop plants and Vegetables:*

Though the phenomenon of hybrid vigour or heterosis has been unconsciously exploited by man for the improvement of plants and animals since ancient times, it is only after the discovery of the laws of genetics in the early years of the present century that the conscious exploitation of this phenomenon started. At first, the utilization of hybrid vigour was considered profitable only in a cross-pollinated plant like maize; Dr. Pal, however, showed as early as 1932, that hybrid vigour is manifested even among crosses in self-pollinated cereals like wheat. Hence, in wheat breeding programmes, he introduced the concept of evaluating the combining ability among the parents used in crosses. He also showed how hybrid vigour can be commercially exploited in vegetables such as brinjal, *bhindi*, bitter gourd and chillies. Among the other crops studied by him from this point of view are maize, *Sesamum* and gram. Thanks largely to his efforts, an All-India Co-ordinated programme for the exploitation of hybrid vigour in maize is now in operation with headquarters at the Indian Agricultural Research Institute.

*Distant Hybridization:*

Dr. Pal has been a pioneer in the field of carrying out a comprehensive and constant search for new genes among the wild relatives of our crop plants. As a result, a very good collection of the wild relatives of several crop genera has been built up at the Indian Agricultural Research Institute. After evaluating the material for the useful genes they possess, Dr. Pal with his associates has undertaken a planned inter-specific hybridization programme in the genera *Triticum*, *Solanum*, *Lycopersicon*, *Nicotiana* and *Luffa*. By crossing the cultivated tomato species *Lycopersicon esculentum* with a wild south American species *L. pimpinellifolium*, the tomato variety Pusa Red Plum was evolved. This variety is rich in Vitamin C and has a high sucrose content. It resembles a plum in appearance and is growing in popularity among kitchen

gardeners. Many potato varieties possessing resistance to the late blight fungus were also evolved from inter-specific crosses.

During the course of a search for useful genes among wild relatives of crop plants, a species belonging to the same genus as the cultivated *bhindi* was found to possess resistance to the yellow-vein mosaic virus, which causes a severe damage to this vegetable crop throughout India. On a closer study this species was found to be a new one, not corresponding to any that has already been described. Dr. Pal and Mr. H. B. Singh named this species *Abelmoschus tuberculatus* and subsequent cytogenetical research has fully justified the specific status assigned to this species and in addition, has revealed that this species is one of the probable ancestors of the cultivated *bhindi*, *A. esculentus*.

*Plant Breeding Techniques:*

The development of modern techniques has converted plant breeding from an art into a science. Dr. Pal's contributions in the field of developing and applying suitable techniques in plant breeding work have been many. Among them, mention may be made of his work on vernalisation and photoperiodic treatment to bring about synchronisation of flowering in varieties and species of *Triticum*, induction of polyploidy in crop plants by colchicine treatment and the application of statistical techniques such as ' Discriminant function ' in selection procedures.

*Breeding improved varieties of crop plants other than wheat:*

Dr. Pal has initiated and guided plant breeding work designed to evolve superior varieties of several other crop plants besides wheat. Among them, special mention may be made of the new varieties of potato and hookah tobacco produced by him in collaboration with some of his colleagues.

*Induction of mutations in plants through the use of atomic radiations:*

The advent of atomic energy has provided new and elegant research tools; Dr. Pal has initiated at the Indian Agricultural Research Institute a comprehensive programme to exploit to the full the potentialities offered by atomic energy in agricultural research. In this, he has been assisted by an enthusiastic band of researchers. He gave an outline of the results achieved under this programme at a Symposium on " Radioisotopes " organised by the National Institute of Sciences of India in May, 1957, at Bombay. His own particular interest has been in the use of radioisotopes to induce specific mutations in desirable genotypes. Thus, the character of awning was introduced into the wheat variety N.P. 809 by $P^{32}$ treatment. This is an extremely important achievement since Indian farmers prefer awned varieties in the belief that the presence of awns prevents damage to grains by birds. The same objective can be achieved by hybridization followed by backcrossing but this many times leads to a dilution of desirable genotypes. Dr. Pal has, thus, shown that wherever possible, specific gene changes should be brought about by mutation research without disturbing ideal gene combinations. In order to intensify the mutation research programme at the I.A.R.I., he took a prominent part in setting up a Cobalt-60 field radiation unit at the Institute. Dr. Pal is the Chairman of the Special Advisory Committee on Food and Agriculture set-up by the Department of Atomic Energy.

*Genetical studies:*

Besides a detailed investigation of the genetics of rust resistance and other characters in wheat, Dr. Pal has carried out a study of the self- and cross-incompatibility mechanism in diploid tuber-bearing *Solanum* species and has shown that the oppositional allelic system controls the incompatibility reaction in this group. He further

showed that bud pollination might help to overcome self-sterility in some *Solanum* species. Another important finding is that sex determination in the genus *Luffa* is genically controlled.

*Other achievements:*

The collection and breeding of ornamental plants have been Dr. Pal's major hobby. He has a fine collection of roses and has evolved several new varieties of *Bougainvillea*. His book on "Indian Climbers" has been published by the Indian Council of Agricultural Research. Dr. Pal has been deeply interested in the application of the results of agricultural research for the benefit of the farmers. Under his over-all guidance, the Indian Agricultural Research Institute has taken up the task of demonstrating to the farmers in several villages adjoining Delhi what improved methods can achieve. He has made a detailed study of "Land Transformation" problems. He has also taken an active interest in popularising the use of improved varieties of vegetables. To provide good seeds of the improved varieties to the farmers, he has initiated seed multiplication and testing programmes. In furtherance of his abiding interest in the Plant Kingdom as a whole, he has encouraged research work on algae and other lower plants and has himself written (jointly with others) a monograph on "Indian Charophyta" in a series of monographs on Indian algae sponsored by the Indian Council of Agricultural Research.

During the last 10 years, Dr. Pal has discharged with great distinction the heavy responsibilities of the Director of the Indian Agricultural Research Institute. The Institute has undergone considerable expansion during this period and from the point of view of the scope of the research problems now tackled at the various Divisions of the Institute and the extent of facilities available for research work both in terms of trained personnel and specialised equipment, the Institute has been transformed beyond recognition. Due to Dr. Pal's efforts, the Institute has been vested with the authority of a University and regular M.Sc. and Ph.D. courses were commenced in October, 1958. The popularity of the I.A.R.I. Post Graduate School will be obvious from the fact that more than 1,000 candidates have been applying for admission each year. That he has, amidst his various administrative and advisory duties, always found time both to participate actively in the research projects which he had initiated and to start new lines of investigation serves as an index of Dr. Pal's interest and devotion to genetical research. As the Chairman of the Botany Committee of the Indian Council of Agricultural Research for the past many years, Dr. Pal has played a dominant role in giving to the science of genetics the stature it now enjoys in India in the field of biological research.

The members of the Indian Society of Genetics and Plant Breeding are proud and happy that, through the award of the Rafi Ahmed Kidwai Prize, Dr. Pal's contributions to the development of these sciences in India have received recognition in yet another way. It is their fervent hope and wish that Dr. Pal may continue to guide and inspire genetical research in India for many years to come.

(M. S. Swaminathan)

# A NEW METHOD OF HYBRIDIZATION IN COTTON

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The utility of interspecific and other remote crosses in breeding improved types is well recognized. Extensive research aimed at increasing the degree of success in such wide crosses has been undertaken in many countries. Investigations along these lines in the Soviet Union have brought out the favourable influence on plant growth of using a mixture of pollen, the influence of the amount of pollen in the process of fertilization and the important role played by the carotin, carotinoids, enzymes and other biologically active substances present in considerable quantities in the pollen. It has also been shown that egg cells which have been fertilized by a sperm and have begun to divide can be affected by other male gametes. Thus, by successive pollination, first with unlabelled and subsequently with $P^{32}$ and $S^{35}$ labelled pollen, Polyakov and Dmitrieva (1955) were able to show, in tobacco, makhorka and corn, that the labelled pollen also took part in the process of fertilization by influencing some metabolic processes. Pollen from an alien species was also shown to have a definite influence. The technique of radioisotope labelling is of interest to us here in that it enables us to determine the number of pollen tubes which discharge their contents into one ovule during the process of fertilization. Preliminary experiments indicate that the number is about 3-8 per ovule. The results of these authors agree with our empirical conclusion reached on the basis of several years' experience as to the influence of the application of varying quantities of pollen in hybridization work.

## Materials and Methods

The experiments reported in this paper were carried out at the Central Asia Experiment Station of the All-Union Institute of Plant Industry near Tashkent in the Uzbek Soviet Socialist Republic in 1949. Two species of cotton, *Gossypium hirsutum* and *G. herbaceum*, which are known to be difficult to cross, formed the experimental material. The variety of *G. hirsutum* chosen, C-460, is a late maturing type, 50-60 per cent of the total yield being available before the first autumn frosts. It has a staple length of 31-32 mm., a ginning out-turn of 40-41 per cent, and a boll weight of 7·0-7·5 gm. K-2287, the red-leaved variety of *G. herbaceum* used, is a very late maturing type with a staple length of not more than 24-25 mm., a ginning out-turn of 29-31 per cent and a boll weight of 5·0-5·5 gms. The plants used in the experiment were derived from stocks which had been self-pollinated for two years. Buds of C-460 were emasculated the evening before they were to open and isolated. The next morning, the emasculated buds were pollinated with a restricted amount of pollen (about 20 grains) of C-460 and three hours later an unlimited amount of K-2287 pollen was applied to the stigmas of the same flowers. The second treatment consisted of the application of about 20 pollen grains from the variety K-2287 to non-emasculated flowers of C-460 in the morning. Emasculated and non-emasculated flowers of C-460 pollinated with unlimited amounts of K-2287 pollen served as controls.

## Results

The seeds obtained from the two treatments and one* of the controls were sown separately in the spring of 1950. The observations recorded on these may be summarised as follows:

All the plants raised from the control (unlimited K-2287 pollen applied in the morning to stigmas of non-emasculated flowers of C-460) as well as one of the treatments, (where unlimited amounts of K-2287 pollen were applied to stigmas of non-emasculated flowers of C-460, 3 hrs. after opening) resembled the maternal parent completely. However, where the application of a limited amount of the maternal pollen was followed by the application, after 3 hours, of an unlimited amount of pollen of the paternal variety K-2287, a number of plants clearly differing morphologically from C-460, and possessing a number of the morphological characteristics of K-2287, were obtained. A short morphological and agronomical description of the parents and the hybrid-seedlings is given below:

A. *G. hirsutum* L. var. C-460. Bush with relatively open habit; 100-200 cm. tall; fruiting shoots sympodial; leaves broad, five lobed, green, with entire margins; bolls large, orbicular-oval in form, five loculed with an acute top which forms a well-shaped rostellum.

B. *G. herbaceum* L. var. K-2287. Open bush, 135-145 cm. tall, spreading; fruiting spurs thin, trailing. All parts sparsely pubescent; leaves five lobed, the lobes being oval, narrow at the base with an obtuse tip; Bolls large, 4-5 loculed, orbicular, flattened at the rostellum with a clearly shaped star; bracts with 5-8 teeth.

C. The hybrid seedlings could be classified into the following six groups.

(i) Compact bushes, 60-70 cm. tall with erect stems, pubescent; leaf lobes slightly orbicular, narrow at the base as in the K-2287 parent; bolls large, 4-5 loculed, orbicular in shape (with more or less evident star) shallow pitting on the surface; bracts with 7-9 teeth as in the K-2287 parent; cotton firmly held in the locules; plants fruitful, early maturing. Only a few plants fall into this group.

(ii) Similar to (i) except that the bushes are more compact and earlier maturing.

(iii) Pyramidal bushes, 80-90 cm. tall; fruiting shoots sympodial; stem and leaves sparsely pubescent, the leaves falling off when the bolls mature; bolls large, 4-5 locular, globular in shape, shallow pitted on the surface and flattened as in the K-2287 parent; bracts 7-8 toothed; early maturing and high yielding.

(iv) Resembling K-2287 very much in habit, the leaves and fruits on the lower fruiting branches being shed; stem and leaves pubescent; bolls orbicular, 4-5 loculed.

(v) Well-shaped bushes, a metre or more in height; monopodial, short fruiting spurs; stem erect; leaves large; bolls oval with elongated rostellum; medium late in maturity and fruitful.

The staple length in the hybrid seedlings ranged between 26-33 mm. and the ginning outturn between 30 and 38 per cent. Plants in the first three groups matured 13-18 days earlier than the C-460 parent.

In the next season, the progeny of each group was sown separately in decimeter plots at the rate of 50 plants per plot. Analysis of this generation showed that the typical characters of each group were maintained. Some variation in quantitative characters (yield, staple length etc.), pointed to the influence of the pollen from a different species of cotton on the hereditary characters of the plants obtained.

*All the emasculated buds of C-460 pollinated with an unlimited amount of K-2287 pollen fell off.

Families of value from the breeding point of view were obtained from the third generation (Table 1). Some of the more desirable families have proved to be earlier by 12-18 days than the standard variety 108F, in varietal trials over four seasons and appear suitable for testing in the northern limits of the cotton growing region.

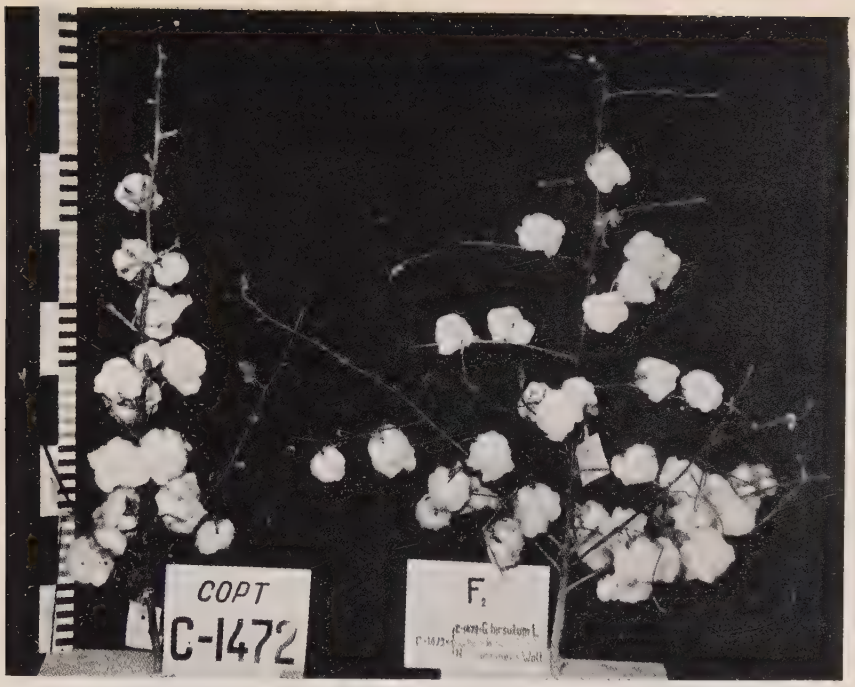
TABLE 1

*The characters of parental types and hybrids in an interspecific cross in cotton*

| Varieties or families | Number of days from sowing-ripening | Approximate height (in cm.) | Boll weight (in gm.) | Yield per plant (in gm.) | Remarks |
|---|---|---|---|---|---|
| Original forms of C-460 *G. hirsutum* L. | 150 | 87·1 | 7·2 | 70·6 | Maternal form |
| K-2287 *G. herbaceum* L. | 140 | 115·2 | 3·3 | 24·0 | Paternal form |
| Family 1 .. | 132 | 64·3 | 5·9 | 43·8 | Plants are short, the fibre is attached to the sides of the boll |
| „ 2 .. | 129 | 63·9 | 5·9 | 51·8 | –do– |
| „ 3 .. | 133 | 70·1 | 5·7 | 44·5 | –do– |
| „ 4 .. | 132 | 62·3 | 6·2 | 38·6 | Plants are early maturing |
| „ 5 .. | 129 | 69·5 | 5·9 | 75·3 | short, fruitful |
| „ 6 .. | 133 | 73·0 | 6·7 | 67·6 | –do– |
| „ 7 .. | 133 | 83·5 | 6·9 | 62·9 | Plants maturing early, of medium height, fruitful, have big bolls |
| „ 8 .. | 129 | 70·3 | 6·7 | 100·0 | –do– |
| „ 9 .. | 134 | 96·2 | 8·2 | 85·3 | –do– |
| „ 10 .. | 129 | 81·4 | 5·8 | 73·2 | –do– |

It is evident from what has been said above that, in wide crosses, applying a limited amount of the maternal pollen before pollinating with the pollen from the paternal form can give rise to interesting and desirable breeding material. In the present case, hybridization of *G. hirsutum* with Old World cotton has led to the appearance of new types combining the characters of the maternal and paternal forms.

In 1952, the same technique was adopted in crosses, with different varieties of *G. arboreum* L. as the pollen parent. Segregants could be selected in the $F_2$ and $F_3$ possessing some of the characters of *G. arboreum* such as small, elongated bolls, narrow bracts with few (3-4) teeth etc. In the progeny, there were early maturing as well as late maturing, fruitful and compact plants. These " acquired characters " were maintained in the progeny of selected plants, indicating the hereditary nature of the variability observed in these cases.

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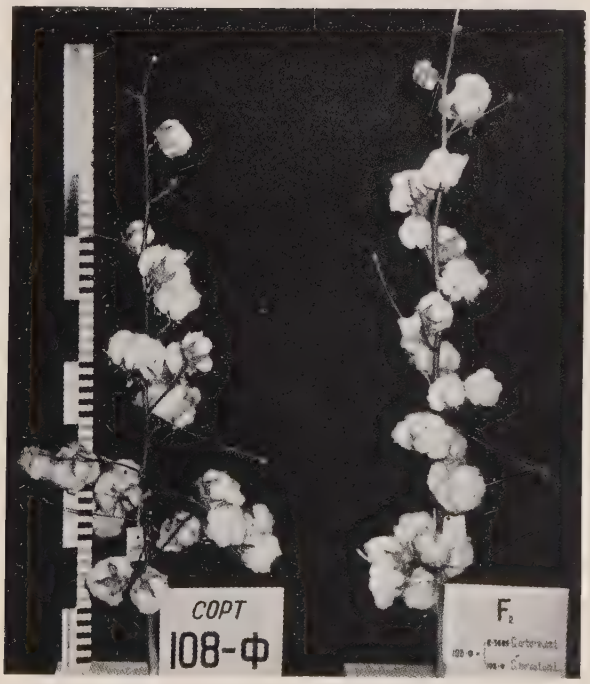


2

FIG. 1. (Left)–variety C–1472 (*G. hirsutum* L.), (right)–$F_2$ of C–1472 × *Hibiscus coccineus* Walt.
FIG. 2. (Left)–variety 108–F (*G. hirsutum* L.), (right)–$F_2$ of 108–F × *G. arboreum* L. (K–3699).

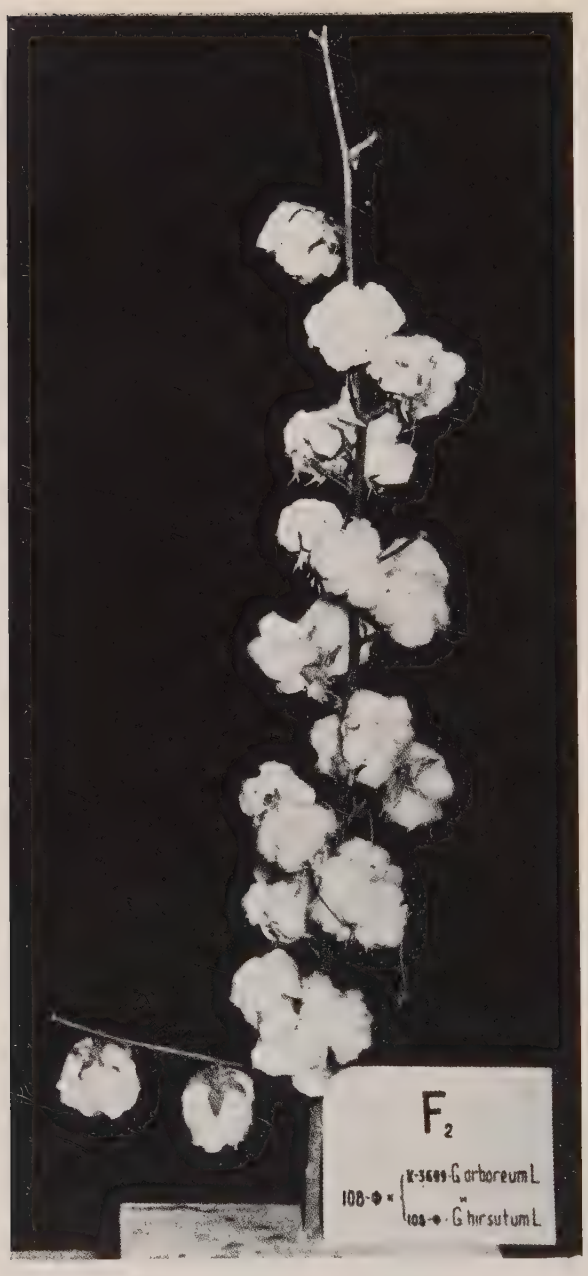


FIG. 3. $F_2$ of 108–F × *G arboreum* L. (K–3699). A new very promising long stapled variety.

In further experiments, three other malvaceous plants—*Hibiscus coccineus*, *H. rosa-sinensis* and *Malva neglecta*, were used as pollen parents on the agronomic cotton variety 108F. 10-20 pollen grains of the maternal parent were applied to the stigmas of emasculated flowers of 108F, followed by successive pollination, 3 hours later, of the same stigmas, with unlimited pollen of the paternal variety. In the progeny were obtained, individuals with poor or good leaf-development, vigorous development of the monopodial branches etc. Similar results were obtained when other varieties were taken as the maternal parent.

The technique was slightly modified in 1955, taking into account the fact that 300-800 or more flowers and buds may be produced on a plant in the growing period. In order to increase the flow of nutrient material to the crossed flowers, all but 2-3 buds on 7-8 fruiting branches were removed from the mother parent. The extra buds were removed from both the sympodial and from the monopodial branches. This resulted in marked changes in the morphology of the treated plants, as compared to the control row of plants, about 35-40, where the buds were not removed. The length and thickness of the main stem increased in the treated plants, the leaf blades were twice as large. In addition to the method of successive pollination described before, another method worked out by the author and his assistant N. Anziferov was also used. In this method, the emasculated buds were immediately pollinated the same evening, with an unlimited amount of pollen from the male parent. Again, an unlimited amount of paternal pollen was applied next day early in the morning followed, at 12 noon (about 20 hrs. after emasculation), by a mixture of equal parts of maternal and paternal pollen. Non-emasculated flowers were taken as controls. The variety used as the maternal form, C-1472 (*G. hirsutum*) is a fruitful, late maturing type with a staple length of 34-35 mm., ginning index of 37-39 per cent and a boll weight of 6·8-7·0 gm. Varieties of *G. herbaceum* L. (K-894) from India, (K-590) from Iran, (K-2282, K-2585) from Uzbekistan and of *G. arboreum* L. from India (K.922, K-3687), Korea (K-1565) and Japan (K-2231) were used as pollen sources. In addition to these Old World cottons, other malvaceous genera were also used.

The original technique—pollinating first with a few pollen grains of the maternal parent followed by the application, 3 hours later, of unlimited quantities of the pollen from the paternal form—gave a fruit set of 60-83 per cent when most of the buds were removed, whereas, if no buds were removed, the fruit set was 5-22 per cent. Pollinating twice with the paternal pollen, followed by the application of maternal and paternal pollen, mixed in equal parts, resulted in a boll set of 93-100 per cent in plants where most of the buds had been removed, whereas untreated plants showed only 13 per cent fruit set. Thus, the removal of all buds except those to be used in hybridisation, from the maternal plant results in a marked increase in the percentage of boll-set, especially on pollination, initially, with a limited amount of self-pollen, followed 3 hours later by an unlimited amount of pollen from another species or even genus. A number of interesting plants have been raised from seeds obtained by this method. As is well known, hybridisation between American and Asiatic cottons is difficult, one hybrid being obtained from several thousand cross-pollinated flowers. Using our technique, however, a typical interspecific hybrid with sterile flowers has been obtained from only 30 flowers of the *G. hirsutum* variety C-1472 pollinated with pollen from K-590, the *G. herbaceum* type from Iran. Adopting the same technique, progeny markedly different from the maternal plant have been obtained in the inter-generic cross *G. hirsutum* (C-1472) × *Hibiscus coccineus*, (Tables 3, 4). When carried to the $F_2$ generation, each plant progeny continued to show the distinguishing characters of the $F_1$ plant and matured 9 days earlier than the maternal forms.

It is not possible, for reasons of space, to describe all the hybrids obtained in crosses involving different varieties of cotton as well as other genera of the family Malvaceae.

TABLE 2

*Some characters of plants, deviating from the maternal type, in the $F_2$ of some inter-specific hybrids in cotton*

| No. of strain | Origin of strain | Name of paternal species | Number of plants | Days to maturity | Boll weight (in gm.) | | Fibre length (in mm.) | | Fibre yield (in %) | | Cotton yield per plant (gr.) | | 1,000 seed weight (gr.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | M. | ±m. | M. | ±m. | M. | ±m. | M. | ±m. | M. | ±m. |
| | Variety 108F selfed | *G. hirsutum* L. | 30 | 136 | 6·6 | 0·15 | 32·2 | 0·34 | 36·9 | 0·38 | 62·4 | 4·26 | 124·7 | 2·58 |
| Str.-4020 .. | 108F × 3699 | *G. arboreum* L. | 30 | 129 | 5·6 | 0·10 | 32·0 | 0.36 | 36.3 | 0·19 | 65·2 | 2·48 | 110·8 | 1·77 |
| Str.-4102 .. | –do– | ,, | 30 | 131 | 6·1 | 0·11 | 33·6 | 0·29 | 35·8 | 0·28 | 77·8 | 4·66 | 116·0 | 1·23 |
| Str.-4021 .. | 108F × K-922 | ,, | 30 | 134 | 4·7 | 0·09 | 30·1 | 6·39 | 34·7 | 0·25 | 63·5 | 4·36 | 108·3 | 1·62 |
| Str.-168 .. | 108F × K-590 | *G. herbaceum* L. | 30 | 141 | 7·8 | 0·14 | 37·1 | 0·29 | 34·4 | 0·23 | 73·2 | 8·21 | 137·0 | 2·36 |
| Str.-169 .. | –do– | ,, | 30 | 142 | 7·7 | 0·12 | 36·7 | 0·32 | 32·9 | 0·20 | 70·0 | 4·66 | 148·1 | 1·73 |

TABLE 3

*The characters of plants deviating from the maternal type in the $F_2$ of the intergeneric cross G. hirsutum* L. (*C*-1472) × *H. coccineus Walt.*

| Characters | Check variety, C-1472 (Mean value for 30 plants) | Deviant segregants (Mean value for 30 plants) | Deviation from the mean of check | Degree of accuracy (t) |
|---|---|---|---|---|
| Length of growing period (in days) .. .. | 136 | 127 | .. | .. |
| Height (in cm.) .. .. | 75·4 | 69·1 | –6·3 | 2·20 |
| Type of bush .. .. | compact | friable | .. | .. |
| Length of the 5th sympodial branch (in cm.) .. | 7·1 | 23·9 | +16·8 | 13·16 |
| Leaf breadth of the 14th node (in cm.) .. .. | 13·9 | 9·2 | –4·7 | 9·17 |
| Leaf length of the 14th node (in cm.) .. .. | 10·4 | 7·9 | –2·5 | 11·06 |
| Boll shape .. .. | egg-shaped | ellipse-shaped | .. | .. |
| Boll surface .. .. | knobby | smooth | .. | .. |
| Seed fuzz .. .. | thick | thin | .. | .. |
| Weight of raw cotton per boll in gm. .. .. | 7·0 | 3·6 | –3·6 | 30·41 |
| Fibre length (in mm.) .. | 34·1 | 25·1 | –9·0 | 18·65 |
| Fibre yield (in %) .. | 38·5 | 34·7 | –3·8 | 8·11 |
| Raw cotton yield per plant (in gm.) .. .. | 72·3 | 47·9 | –24·4 | 3·97 |
| Absolute seed weight (in gm.) | 136·2 | 89·9 | –46·3 | 19·07 |

Some test plants in $F_1$ obtained from crossing with *H. coccineus* Walt. are shown in Table 4.

It must, however, be pointed out that not all these crosses gave segregants likely to be useful in a short-term breeding programme.

Varietal differences in response to such reiterated pollinations were also noted. Thus, the early maturing variety K-4112 as the female parent could not be successfully crossed with *G. herbaceum* and *G. arboreum* though the application of pollen from *Althea rosea* and *Abelmoschus esculentus* resulted in progeny sharply deviating from K-4112. On the other hand, pollinating the variety 108F with pollen from *G. herbaceum* L. and *G. arboreum* L. resulted in progeny differing in morphological and other characters, these changes being maintained in the subsequent generation (Table 2). Selection among such plants has resulted in agronomically valuable strains.

## SUMMARY AND CONCLUSIONS

1. Biochemical and physiological studies have shown that preliminary pollination with a limited amount of pollen from the maternal form causes an activation of

TABLE 4

*The characters of the maternal parent, C-1472, and some $F_1$ hybrids, C-1472 × Hibiscus coccineus*

| Plant characters | Maternal form (variety C-1472) | Test plant |
|---|---|---|
| Height (in cm.) .. .. | 90 | 70 |
| Type of bush .. .. .. | compact | friable |
| Number of monopodial branches .. | 2 | 4 |
| Length of the 5th sympodial branch (in cm.) .. .. .. | 6 | 15 |
| Breadth of leaf at the 15th node (in cm.) | 19·5 | 9·5 |
| Length of leaf at the 15th node (in cm.) | 13·5 | 8 |
| Mean weight of raw-cotton in one boll (in gm.) .. .. .. | 6 | 4 |
| Fibre length (in mm.) .. .. | 34 | 26 |
| Colour of seed fuzz .. .. | grey | green |
| Absolute seed weight (in gm.) .. | 148·9 | 108·4 |

the metabolic processes resulting in an increased inflow of nutritional material as well as other substances into the style and stigma.

2. Such preliminary pollination with limited amount (about 20 grains) of pollen from the maternal species followed by the application, 3 hours later, of pollen from the paternal species resulted in the production of plants, with new combinations of characters, which form valuable breeding material.

3. The application of pollen from the paternal parent in unlimited quantity immediately after emasculation and again the next morning, followed by a mixture of equal parts of maternal and paternal pollen on the third day also resulted in progeny exhibiting a combination of the characters of the two parents.

4. The removal of all but few buds to be used in hybridisation from the maternal parent increases the percentage of boll set and, thus, increases the possibility of getting hybrids.

5. Usually, when a mixture of pollen from two different species is applied to the stigma of one of the species, the pollen of the maternal parent successfully effects fertilization whereas the alien pollen is unsuccessful. The technique described by us has, however, given different results.

6. Under natural conditions, pollen grains (whether of the same species or from alien species or even different genera) once they reach the stigma are able to affect the characters of the progeny. Presumably they do so by influencing the formative processes in the plants.

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# MONOSOMIC ANALYSIS IN WHEAT V. IDENTIFICATION OF CHROMOSOMES CARRYING GENES FOR RESISTANCE TO TWO RACES OF STEM RUST IN THE VARIETY N.P. 790

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Breeding of rust resistant varieties is a complex problem, since the breeder is confronted with two variable biological organisms which interact with each other differently under different environmental conditions. In view of this, Stakman (1954) has stressed the need for collecting exact information on the genetic potentialities for resistance in different wheat varieties. Genetic studies in polyploid plants, such as bread wheat, are complicated by the presence of several factors with complementary, compensatory or inhibitory effect controlling each character. Other minor or modifying genes also render the correct classification of individual phenotypes difficult. The establishment of the monosomic series in bread wheat by Sears (1939, 1944) has, however, resolved this handicap. The usefulness of aneuploids in determining the mode of inheritance of rust resistance in varieties of bread wheat has already been demonstrated by Heyne and Livers (1953), Plessers (1954), Wiggin (1955), Sears and Rodenhiser (1948), Sears *et al.*, (1957), Campbell and McGinnis (1958), Unrau *et al.*, (1958), Knott (1959) and Singh and Swaminathan (1960). The present study was undertaken to locate, by monosomic analysis, the chromosome or chromosomes of the wheat variety N. P. 790 on which genes for resistance to race 21 and 15c of stem rust (*Puccinia graminis tritici* (Pers.) Erikss. and Henn.) are situated. These two races are widely prevalent and are very virulent and as such are of importance in wheat breeding work.

## Materials and Methods

All the twenty one monosomic lines (material obtained from Dr. E. R. Sears), were identified and then crossed with N.P. 790. Identification of monosomes was done by counting the chromosome number in microsporocytes. Normal Chinese Spring was tested for reaction for all the races of stem rust and was found to be susceptible, showing type 4 pustules. For recording pustule type, the standard key drawn up by Stakman, Levine and Loegering (1944) and Stakman and Harrar (1957) was followed. N.P. 790, which was used as the male parent is highly resistant to all the races and biotypes of stem rust (eg., 15, 15c, 17, 21, 21A, 24, 34, 40, 42, 42B, 72, 75, 117, 194) with the exception of race 122 and as such is a valuable source of resistance to wheat breeders in India. 15c which is included in the present investigation is only a biotype of race 15 (Gokhale, 1950). It is a very virulent biotype and most of the varieties like Thatcher, Gabo, Ridley and Kenya E. 220, which are resistant to the other races of black rust, have been attacked by race 15c. The $F_1$ plants of Chinese Spring monosomes $\times$ N.P. 790 were sown in October, 1958 and in March, 1959 monosomes from each line were identified and seeds from twenty confirmed monosomic lines were harvested separately. In January-February 1960, seedling tests of the different $F_2$ progenies were carried out in the glass house of the Botany Division, I.A.R.I. The initial inoculum for both these races was obtained from the Division of Mycology and was multiplied on two susceptible varieties, C591 and Agra Local. One set of seedlings was inoculated with one race at a time. In each case a primary leaf of one week old seedling was inoculated. As a check, a susceptible

variety, Agra Local, the resistant male parent, N.P. 790, and the susceptible female parent Chinese Spring were also inoculated with each set of seedlings under the same conditions of temperature, humidity and light (temperature range, 65°-85°F). When the material was ready for recording after 15-20 days of inoculation, each seedling was classified according to the type of pustule. Each race was taken up separately and the recording was checked twice.

## Experimental Results

*Race* 15*c*—The $F_1$ seedlings of the cross between N.P. 790 and normal and monosomic plants of Chinese Spring were all susceptible to race 15c of *Puccinia graminis tritici*. The $F_2$ progenies derived from monosomic $F_1$ plants of 20 different lines were screened for resistance in the seedling stage and the segregations observed are summarised in Table 1. The $F_2$ progeny of normal Chinese spring × N.P. 790

Table 1

*Segregation for resistance to Race* 15*c in the $F_2$ progenies of Chinese Monosomes × N.P.* 790

| Cross | Total No. of seedlings | Resistant | Susceptible | | Deviation (Expected ratio 15S : 1R) | |
|---|---|---|---|---|---|---|
| | | | Semi-Resistant | Susceptible | $X^2$ | P |
| Ch. Normal × N.P. 790 | 158 | 13 | 21 | 124 | 1·04 | ·50–·30 |
| M2 × N.P. 790 .. | 182 | 11 | 49 | 122 | 0·01 | ·95– |
| M3 × N.P. 790 .. | 112 | 8 | 28 | 76 | 0·15 | ·70–·50 |
| M4 × N.P. 790 .. | 195 | 12 | 57 | 126 | 0·004 | ·95–·80 |
| M5 × N.P. 790 .. | 101 | 8 | 31 | 62 | 0·47 | ·50–·30 |
| M6 × N.P. 790 .. | 149 | 11 | 39 | 99 | 0·32 | ·70–·50 |
| M7 × N.P. 790 .. | 144 | 12 | 50 | 82 | 1·06 | ·50–·30 |
| M8 × N.P. 790 .. | 140 | 14 | 48 | 78 | 3·36 | ·05–·02 |
| M9 × N.P. 790 .. | 111 | 7 | 47 | 57 | 0·00 | ·99– |
| M10 × N.P. 790 .. | 159 | 12 | 56 | 91 | 0·46 | ·50–·30 |
| M11 × N.P. 790 .. | 135 | 8 | 47 | 80 | 0·02 | ·95–·80 |
| M12 × N.P. 790 .. | 102 | 8 | 28 | 66 | 0·40 | ·70–·50 |
| M13 × N.P. 790 .. | 130 | 10 | 40 | 80 | 0·46 | ·50–·30 |
| M14 × N.P. 790 .. | 218 | 136 | 41 | 41 | Very high deviation | |
| M15 × N.P. 790 .. | 186 | 45 | 46 | 95 | High deviation | |
| M16 × N.P. 790 .. | 201 | 118 | 29 | 54 | Very high deviation | |
| M17 × N.P. 790 .. | 146 | 12 | 61 | 73 | 0·96 | ·50–·30 |
| M18 × N.P. 790 .. | 168 | 12 | 50 | 106 | 0·23 | ·70–·50 |
| M19 × N.P. 790 .. | 128 | 10 | 36 | 82 | 0·53 | ·50–·30 |
| M20 × N.P. 790 .. | 145 | 9 | 48 | 88 | 0·00 | ·99– |
| M21 × N.P. 790 .. | 128 | 8 | 48 | 72 | 0·00 | ·99– |

was also tested. Among the $F_2$ plants some were clearly resistant, some clearly susceptible and some had an intermediate type of reaction (2-3 and 3 types of pustules).

When the intermediate and clearly susceptible types are pooled together, a 15 susceptible: 1 resistant ratio is obtained in all the lines excepting those involving chromosomes XIV, XV and XVI. The $F_2$ progenies of $F_1$ plants monosomic for chromosomes XIV and XVI showed the greatest skewness from the 15:1 ratio. These may hence be considered as the "critical monosomic" lines. Rao, Agrawal and Joshi (1960) have recorded the presence of two duplicate recessive genes conferring resistance to race 15c in N.P. 790 from genetic studies in the progenies of crosses between N.P. 790 and C. 273 and C. 518. Our results also suggest a similar interpretation. However, it is difficult to understand the occurrence of such a high frequency of resistant plants in the $F_2$ progenies of $F_1$ plants monosomic for chromosomes XIV and XVI, since a disturbance of the $F_2$ ratio in the critical monosomic lines could happen only if the genes are effective when hemizygous.

Assuming that two duplicate genes $R_1$ and $R_2$ cause susceptibility to race 15c in Chinese Spring, the genotypes of Chinese Spring and N.P. 790 will be **$R_1$ $R_1$ $R_2$ $R_2$** and **$r_1$ $r_1$ $r_2$ $r_2$** respectively. The $F_1$ genotype will be **$R$ $r_1$ $R_2$ $r_2$** and only the double recessive will be resistant in the $F_2$. If **$R_1$** and **$R_2$** are located on chromosomes XIV and XVI respectively, the genotypic constitution of the $F_2$ progenies derived from $F_1$ plants monosomic for chromosome XIV will be as follows (though the $F_2$ progeny will consist of plants with 2n = 41, 42 and 40 in the relative frequency of 73 per cent, 24 per cent and 3 per cent respectively, (*see* Sears, 1959), the ratios given below have been worked out without taking the nullisomes into consideration since they occur at very low frequencies).

| Chromosome No. | Genotype | Frequency | Expected rust reaction |
|---|---|---|---|
| 2n = 41 | **$R_2$ $R_2$ $r_1$** | 3 | Susceptible |
| ,, | **$R_2$ $r_2$ $r_1$** | 6 | ,, |
| ,, | **$r_2$ $r_2$ $r_1$** | 3 | Resistant |
| 2n = 42 | **$R_2$ $R_2$ $r_1$ $r_1$** | 1 | Susceptible |
| ,, | **$R_2$ $r_2$ $r_1$ $r_1$** | 2 | ,, |
| ,, | **$r_2$ $r_2$ $r_1$ $r_1$** | 1 | Resistant |

Hence, theoretically, the 15:1 ratio will be modified into a 3:1 ratio in the critical monosomic crosses. The $F_2$ plants nullisomic for chromosome XIV will be totally lacking in the **$r_1$** gene. Hence, the occurrence of an excess of resistant plants in the $F_2$ progenies of Mono-XIV and Mono-XVI would suggest that some of the other genotypes may also be resistant. It seems possible that plants of the genotypes **$R_2$ $r$ $r_1$** and **$R_2$ $r_2$ $r_1$ $r_1$** may be partially resistant and this may be one reason why several seedlings showing an intermediate type of reaction occur in all the other lines also. A study of the nature of segregation for resistance occurring in the progenies of seedlings with distinct types of reaction (like 0-2, 2-3 and 3-4) will, however, be necessary before the operation of a gene dosage effect with regard to rust resistance can be considered as definitely established. Besides gene dosage effect, the action of modifiers may also complicate the study of the genetics of rust resistance. While testing substitution lines of the varieties Hope, Red Egyptian, Thatcher and Timstein, Sears *et al.* (1957) inferred that modifiers of resistance may be present in chromosomes other than the critical ones. A similar inference may explain the occurrence of an excess of resistant seedlings in the $F_2$ of the cross Mono XV × N.P. 790.

*Race* 21.—The results obtained with race 21 are summarized in Table 2. From the data, it can be inferred that the cross involving Chinese monosome II × N.P. 790

is the critical line, as it shows the maximum deviation from the expected segregation. In all other lines the observed segregation fits a 3 susceptible:1 resistant ratio, indicating the presence of one gene. The results clearly demonstrate that chromosome II of N.P. 790 carries one recessive gene conferring resistance to race 21.

TABLE 2

*Segregation for resistance to Race* 21 *in the $F_2$ progenies of Chinese Monosomes × N.P.* 790

| Cross | Total No. of Seedlings | Observed | | Deviation (Expected ratio 3S : 1R) | |
|---|---|---|---|---|---|
| | | Resistant | Susceptible | $X^2$ | P |
| Ch. Normal × N.P. 790 | 133 | 36 | 97 | 0·30 | ·70–·50 |
| M2 × N.P. 790 .. | 262 | 230 | 32 | .. | Very high deviation |
| M 3 × N.P. 790 .. | 144 | 34 | 110 | 0·15 | ·80–·70 |
| M 4 × N.P. 790 .. | 137 | 39 | 98 | 0·87 | ·80–·70 |
| M 5 × N.P. 790 .. | 115 | 33 | 82 | 0·84 | ·80–·70 |
| M 6 × N.P. 790 .. | 145 | 37 | 108 | 0·02 | ·90–·80 |
| M 7 × N.P. 790 .. | 124 | 32 | 92 | 0·04 | ·90–·80 |
| M 8 × N.P. 790 .. | 119 | 33 | 86 | 0·47 | ·50–·30 |
| M 9 × N.P. 790 .. | 129 | 33 | 96 | 0·02 | ·90–·80 |
| M10 × N.P. 790 .. | 119 | 26 | 93 | 0·60 | ·50–·30 |
| M11 × N.P. 790 .. | 137 | 27 | 110 | 2·05 | ·20–·10 |
| M12 × N.P. 790 .. | 136 | 37 | 99 | 0·35 | ·70–·50 |
| M13 × N.P. 790 .. | 136 | 34 | 102 | 0·00 | No deviation |
| M14 × N.P. 790 .. | 140 | 38 | 102 | 0·34 | ·70–·50 |
| M15 × N.P. 790 .. | 128 | 33 | 95 | 0·04 | ·90–·80 |
| M16 × N.P. 790 .. | 126 | 37 | 89 | 1·28 | ·30–·20 |
| M17 × N.P. 790 .. | 88 | 22 | 66 | 0·00 | No deviation |
| M18 × N.P. 790 .. | 143 | 35 | 108 | 0·02 | ·90–·80 |
| M19 × N.P. 790 .. | 130 | 35 | 95 | 0·25 | ·70–·50 |
| M20 × N.P. 790 .. | 109 | 32 | 77 | 1·10 | ·30–·20 |
| M21 × N.P. 790 .. | 108 | 29 | 79 | 0·19 | ·70–·50 |

## DISCUSSION

The allocation of the different chromosomes of bread wheat to the different genomes has now nearly been completed and Sears (1959) has proposed a revised system of chromosome nomenclature using the genome symbol as a suffix and homeology as a basis of numerical grouping. According to this system, chromosomes II and XIV are designated as 2A and 1A respectively and chromosome XVI as 3D. We had earlier observed (Singh and Swaminathan, 1960) that genes for rust resistance are more widely prevalent in the A and B genome chromosomes and the present results are also in accord with this view.

It is well known that while the scoring for resistance or susceptibility to rusts according to the types of pustules is a satisfactory method, there are many modifying factors which can affect the manner of reaction. The rigid apportioning of seedlings with intermediate types of reaction to one or the other of two contrasting categories tends to obscure phenomena like incomplete dominance and the existence of a gene

dosage effect where duplicate factors are involved. It will be desirable in genetic studies of rust resistance to take this situation into consideration and try to follow the subsequent behaviour of the different phenotypic classes so as to establish their correct genotypic identity. Finally, the recessive nature of rust resistance in crosses among many varieties of bread wheat provides great possibilities both for carrying out biochemical studies of the nature of resistance and for attempting to induce mutations for resistance in susceptible varieties with wide adaptability and good grain quality. Many of the phenotypically detectable mutations in bread wheat owe their origin to deletions (Swaminathan, 1957). The dominant nature of rust susceptibility may imply that the gene acts either as an inhibitor of a reaction necessary for resistance or as the promotor of a reaction obligatory for the spread of the pathogen. Viewed in either way, cryptic deletions may play a positive role with regard to the introduction of rust resistance in susceptible varieties.

## Summary

Resistance to race 15c of stem rust in the bread wheat variety N.P. 790 appears to be governed by two recessive duplicate factors located in chromosomes 1A and 2A. An excess of resistant seedlings, however, occurs in the $F_2$ lines involving these two chromosomes and the existence of a gene dosage effect with regard to the phenotypic manifestation of disease symptoms is suggested. Resistance to race 21 was controlled by a single recessive gene located on chromosome 3D.

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# KARYOMORPHOLOGICAL STUDIES IN *SORGHUM ANKOLIB* VAR. ANNALIB RED, A EU-SORGHUM

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The importance of sorghum in our agriculture lies in its being the heaviest yielder of both grain and straw among the rainfed cereal crops. The grain serves as the staple food for the poorer classes while the straw makes very good fodder. Inspite of the fact that sorghum plays a very important role in the agriculture of many countries, comparatively little attention has been paid to the study of the fundamental aspects of this cereal. Recently, however, the need for such studies has come to be recognized by sorghum breeders.

Karyomorphological studies, especially at meiosis, yield a wealth of information. The utility of such studies was first demonstrated by McClintock (1929) working with maize. Later work on these lines has, however, been quite scarce and except for a few investigations [*Secale Cereale*, (Lima-de-Faria, 1948, 52); *Lycopersicon esculentum* (Brown 1949; Barton 1950); *Hordeum* (Sarvella *et al.*, 1958) and *Plantago ovata* (Hyde, 1953)] this line of approach has been neglected. Perhaps a major reason for such neglect was the difficulty in obtaining suitable preparations of the earlier stages of meiosis which are most suited for such studies. However, the effort needed to obtain suitable preparations of these earlier stages can prove quite worthwhile. For, the chromosomes at this stage are much less condensed than they are at mitotic metaphase and hence, may show a greater number of morphological land marks which can help in the identification of each individual chromosomes. Furthermore, in as much as the homologous chromosomes are closely paired, the occurrence of even small structural differences in one of them will be clearly brought out at this stage. The analysis of pairing and chiasma formation at this stage can, therefore, supplement considerably observations on these phenomenon at metaphase.

It is the purpose of this paper to present the results of karyomorphological studies on *Sorghum ankolib*, a species belonging to the sub-genus *Eu-sorghum*.

## Materials and Methods

Seeds of *Sorghum ankolib* var. annalib red (I.W. 1105) obtained through the Plant Introduction Section of the Botany Division, Indian Agricultural Research Institute, New Delhi, were sown in the field. For the study of meiotic chromosomes, the simple propiono-carmine smear method (Swaminathan *et al.*, 1954; Magoon *et al.*, 1958) was used. For mitotic studies the root tips from the germinating seeds were pretreated with ·002M 8-hydroxy quinoline for three hours, washed thoroughly with water, fixed in acetic-alcohol (1:3) for 24 hours and stored in 70 per cent alcohol. Before the root tips were crushed under a drop of acetocarmine, they were hydrolysed in 1N HCl at 60° C for 20 minutes, washed thoroughly in cold water and then stained with Feulgen stain prepared according to the schedule of Darlington and Lacour (1947). In such preparations, the metaphase chromosomes, with clearly marked constriction regions, lie widely scattered in a colourless cytoplasm and are quite suitable for an analysis of the karyotype.

Various methods of depicting the pachytene chromosomes have been used, depending mostly on the type of the pachytene chromosomes. In several species the chromosomes have been photographed and straight line drawings made of the various

regions (Barton 1950; Gottschalk 1954 in tomato). Lima-de-Faria (1952) in *Secale* and *Agapanthus* and Sarvella *et al.*, (1958) in Barley, on the other hand, have followed chromomeric patterns and their intensities. Since in *Sorghum ankolib* the pachytene chromosomes proved to be somewhat similar to that of tomato, the former method was found to be the best one to depict the pachytene chromosomes. For the sake of convenience, in comparing the chromosome sizes both for pachytene and mitotic metaphase chromosomes "Relative length", which represents the ratio in percentage of the length of the individual chromosome to that of the longest (Huziwara 1956), was used.

Analysis of the stages of meiosis and mitosis, and photomicrographs of the same, were best made from temporary preparations. Some slides were also made permanent for record purposes following the usual procedures (Swaminathan *et al.*, 1954).

## Results

I. *Mitotic chromosomes.*—Twenty chromosomes are present in the normal somatic complement (Fig. 3). The chromosomes on an average are small ranging from 2·06 to 4·93$\mu$. Depending on the length of the chromosome, centromeric position and presence or absence of secondary constriction, the chromosome complement can be divided into the following six types (Table 2):

Type I:—A pair of long chromosomes (1, 2) generally the longest in the complement, having a secondary constriction on the short arm close to the centromeric position. The centromere is submedian.

Type II:—Two pairs of medium sized chromosomes (3, 4 and 7, 8) having sub-median centromeric constriction.

Type III:—One pair of medium sized chromosomes (5, 6) with median centromeric constriction.

Type IV:—One pair of short chromosomes (17, 18) having sub-median centromeres.

Type V:—One pair of short chromosomes (11, 12). The centromeric constriction is almost sub-terminal.

Type VI:—Four pairs of short chromosomes (9, 10; 13, 14; 15, 16; and 19, 20). All these four pairs have median centromeres.

No satellited chromosomes were observed in the preparations studied.

II. *Morphology of the Meiotic Chromosomes.*—Pre-pachytene stages do not lend themselves to accurate analysis. However, the observations made in several nuclei showed that pairing is initiated at the proximal end and progresses towards the distal end. In late pachytene and early diplotene, the split threads fall apart earlier in the distal segments. The centromere of each of the 10 pachytene chromosomes is readily distinguished as an oval shaped achromatic structure flanked by segments consisting of deeply staining chromomeres. These are followed by lightly staining regions which, excepting in one arm of one chromosome in the complement, exceed the length of the deeply-chromatic regions.

Generally the following characters, alone or in combination, are used to identify the individual pachytene bivalent: (1) relative length, (2) chromomeric patterns, (3) deep staining knobs, (4) position of the centromere, (5) relative length of chromatic and achromatic portions of each arm.

In the present material, neither distinct chromomeric patterns nor differentially staining knobs were noted. Therefore, the recognition of the individual bivalents has been based on the other three factors. Figure 1 shows a typical pollen mother cell at pachytene wherein all the $10_{II}$ can be traced from one end to the other. Based upon the average values obtained by accurate measurements of the chromosomes in

TABLE 1

*The average length in microns of the Pachytene complement*

| Chromosome | Long arm | | | Short arm | | | Total chromosome length | Arm ratio |
|---|---|---|---|---|---|---|---|---|
| | H.S.R.[1] | L.S.R.[2] | Total | H.S.R. | L.S.R. | Total | | |
| I | 5·75 | 38·22 | 43·97 | 2·69 | 31·47 | 34·16 | 78·13 | 1 : 1·3 |
| II | 2·33 | 34·70 | 37·03 | 3·33 | 22·77 | 26·10 | 63·13 | 1 : 1·4 |
| III | 4·60 | 34·57 | 39·17 | 2·66 | 18·79 | 21·45 | 60·62 | 1 : 1·8 |
| IV | 3·33 | 29·11 | 32·44 | 4·12 | 17·81 | 21·93 | 54·37 | 1 : 1·6 |
| V | 3·12 | 19·46 | 22·58 | 2·66 | 16·01 | 18·67 | 41·25 | 1 : 1·2 |
| VI | 4·12 | 29·10 | 33·22 | 5·30 | 1·71 | 7·01 | 40·23 | 1 : 4·7 |
| VII | 5·33 | 18·69 | 24·02 | 3·91 | 10·19 | 14·10 | 38·12 | 1 : 1·7 |
| VIII | 2·66 | 16·24 | 18·90 | 2·12 | 15·85 | 17·97 | 36·87 | 1 : 1·1 |
| IX | 2·66 | 15·50 | 18·16 | 2·12 | 10·97 | 13·09 | 31·25 | 1 : 1·4 |
| X | 2·22 | 12·88 | 15·10 | 2·33 | 10·69 | 13·02 | 28·12 | 1 : 1·2 |

1. H.S.R. = Heavily stained Region.
2. L.S.R. = Lightly stained Region.

TABLE 2

*A comparative study between somatic chromosomes and Pachytene chromosomes*

| Somatic chromosome pairs | Length in $\mu$ | | Relative length | | Arm ratio | | *Centromere position | |
|---|---|---|---|---|---|---|---|---|
| | Somatic | Pachytene | Somatic | Pachytene | Somatic | Pachytene | Somatic | Pachytene |
| 1, 2 | 4·93 | 78·13 | 100 | 100 | 1 : 1·4 | 1 : 1·3 | Sm | Sm |
| 3, 4 | 4·33 | 63·13 | 88 | 80 | 1 : 1 | 1 : 1·4 | M | Sm |
| 5, 6 | 4·26 | 60·62 | 86 | 78 | 1 : 1·8 | 1 : 1·3 | Sm | Sm |
| 7, 8 | 3·60 | 54·37 | 73 | 70 | 1 : 1·4 | 1 : 1·6 | Sm | Sm |
| 9, 10 | 2·60 | 41·25 | 53 | 53 | 1 : 1 | 1 : 1·2 | M | Sm |
| 11, 12 | 2·60 | 40·23 | 53 | 51 | 1 : 3 | 1 : 4·7 | St | St |
| 13, 14 | 2·46 | 38·12 | 50 | 48 | 1 : 1 | 1 : 1·7 | M | Sm |
| 15, 16 | 2·26 | 36·87 | 46 | 47 | 1 : 1·1 | 1 : 1·1 | M | M |
| 17, 18 | 2·13 | 31·25 | 44 | 40 | 1: 1·3 | 1 : 1·4 | Sm | Sm |
| 19, 20 | 2·06 | 28·12 | 42 | 36 | 1 : 1 | 1 : 1·2 | M | Sm |

* Sm = Sub median.
M = Median.
St. = Sub-terminal.

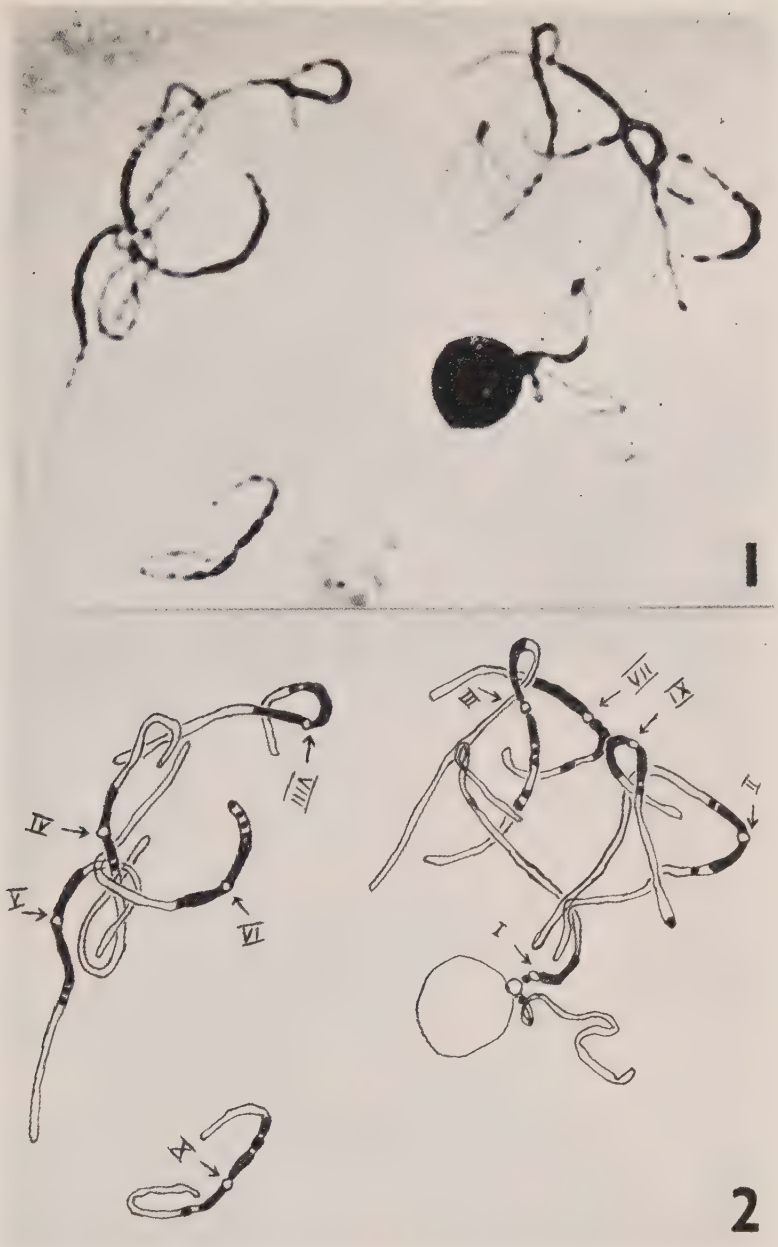


FIG. 1. Pachytene chromosomes of *Sorghum ankolib*. (X1800).
FIG. 2. Diagrammatic representation of pachytene chromosomes of *S. ankolib*. Chromosomes are numbered according to their length.

several nuclei, where all the chromosomes could be clearly traced from end to end, the ten pachytene chromosomes have been arranged in the order of their length and numbered from 1 to 10, chromosome 1 being the longest and chromosome 10 being the shortest. Principal diagnostic morphological features of each of the ten chromosomes in the haploid complement of *Sorghum ankolib* are presented briefly below (Table 1 and 2).

*Chromosome I.*—This is the longest of the complement measuring on an average 78·13 $\mu$; with a nucleolar organizing region (in the short arm) lying very close to the centromere. Another diagnostic feature of this chromosome is the presence of 2 to 3 deeply-stained chromomeres on the short arm just distal to the nucleolar organizing region. The arm ratio is 1:1·3.

*Chromosome II.*—This measures about 63·13 $\mu$, having an arm ratio of about 1:1·4. This and the third chromosome are about equal in their overall length but chromosome II can be distinguished from chromosome III both on the basis of the different arm-ratio and by the presence in chromosome II of two deeply staining chromomeres, one on each arm, terminating the heavily stained segments and a second deeply staining chromomere on the long arm a little away from the first chromomere.

*Chromosome III.*—Measures on an average 60·62 $\mu$ with an arm ratio 1:1·8. The chief feature available for the identification of this chromosome is that the long arm is almost twice the length of the short arm. Also, there are 3-4 deeply staining chromomeres, on the short arm while the deeply staining region on the long arm ends sharply.

*Chromosome IV.*—This measures about 54·37 $\mu$ and has an arm-ratio of 1:1·6. It is, therefore, difficult to differentiate this chromosome from chromosome II on the basis of arm ratio alone. However, the identification of the chromosome is facilitated by the presence of one deeply staining chromomere on the long arm, closely following the deeply stained region. The deeply stained region on the short arm ends abruptly.

*Chromosome V.*—It measures about 41·25 $\mu$ and has an arm ratio of 1:1·2. This can be easily distinguished from the preceding chromosomes on the basis of its comparatively shorter length while its differentiation from the shorter chromosomes is facilitated by the presence of 2 chromomeres on the long arm following the deeply staining region.

*Chromosome VI.*—It measures about 40·23 $\mu$. This is readily identified in the complement by its great asymmetry, the arm ratio being approximately 1:5. Almost $\frac{3}{4}$th of the short arm is deeply stained. Following this darkly stained region, there are 2-3 darkly staining chromomeres and the short arm ends with a well marked terminal chromomere. On the long arm, the darkly staining region ends abruptly and the remaining region is almost nonstained. The other characteristic of this chromosome is that in mid or slightly early pachytene the arm ratio as stated above is 1:5 but in late pachytene the ratio is modified to 1:3 or 1:2·5. The absolute length of the short arm in all stages of pachytene appears to be almost constant. Thus, the shift in the arm ratio at different pachytene stages may be due to the unilateral shortening of the long arm without a proportionate reduction in the length of the short arm.

*Chromosome VII.*—This measures 38·12 $\mu$ and has an arm ratio of 1:1·7. This chromosome can be distinguished from the other short chromosomes by the much greater length of the deeply staining region on the long arm as compared to that on the short arm. In addition to the proximal deeply staining region on the short arm, there is second deeply staining region measuring about 2 $\mu$ and separated from the proximal portion by a lightly staining region. The arm ratio can also be conveniently used in separating this chromosome from the other short chromosomes in the complement.

*Chromosome VIII.*—The chromosome is 36·87 $\mu$ long and has an arm ratio nearly equal to unity. This chromosome can, therefore, be easily picked out from the rest of the complement because of the nearly equal arms. Chromosome X also shows a similar feature but the size differences between them (on an average more than 8 microns) proves very useful in differentiating one from the other. Another factor helping in discriminating between the two is the presence of only one chromomere on the short arm of chromosome VIII.

*Chromosome IX.*—This measures 31·25 $\mu$ and has an arm ratio of 1:1·4. The quick identification of this chromosome among the short chromosomes of the complement is facilitated by the characteristic presence of two chromomeres on the long arm, one immediately next to the deeply staining region, the other almost terminal. The darkly staining region on the short arm ends abruptly.

*Chromosome X.*—This is the shortest chromosome of the complement measuring on an average 28·12 $\mu$. Both the arms are almost equal in length, the arm ratio being 1:1·2. The short arm has two deeply staining chromomeres while the long arm has one deeply staining chromomere distal to the deeply staining region.

Based on these characters the pachytene idiogram of all the 10 chromosomes has been constructed (Figs. 8 and 9) bringing out the diagnostic features of the individual chromosomes which proved particularly useful in their identification in the complement.

III. *Diakinesis and Metaphase I.*—The differentially stained regions can be easily traced from diplotene to the late diakinesis. Chiasmata were observable at early and late diakinesis and also at metaphase I (Figs. 4, 5, 6). A total of 85 cells were analysed for chiasma frequency from early diakinesis to metaphase I (Table 3).

TABLE 3

*Chiasma frequencies from early diakinesis to metaphase-I*

| Stage | No. of cells analysed | Bivalents with | | | | Total No. of xta | Average No. of xta/cell | Average No. of xta/bivalent | Terminalization Co-efficient |
|---|---|---|---|---|---|---|---|---|---|
| | | 3xta | 2xta | lxma | 0xma | | | | |
| Early diakinesis | 20 | 50 | 141 | 9 | 0 | 441 | 22·05 | 2·20 | 0·282 |
| Late diakinesis | 35 | 0 | 275 | 71 | 4 | 621 | 17·74 | 1·77 | 0·523 |
| Metaphase-I .. | 30 | 0 | 134 | 157 | 9 | 425 | 14·17 | 1·42 | 0·785 |

It is quite apparent from the data presented in Table 3 that there is a decrease in the number of chiasmata per nucleus from early diakinesis to metaphase. This is further evidenced by the values of terminalization coefficient at MI given in the last column of Table 3. These values also suggest that the process of terminalization is incomplete. The analysis made at early diakinesis revealed the fact that generally the longer chromosomes form a larger number of chiasmata (i.e. 3xta/bivalent) than the shorter ones (1xma/bivalent). Also, at this stage, in about 50 per cent of the cells, all the chromosomes appeared to have a minimum of 2 chiasmata.

Other meiotic stages appeared to be normal. Rarely, delayed separation of a bivalent and lagging of one or two chromosomes were noticed at AI (Fig. 7).

## Discussion

All cultivated species of *Sorghum* belong to the subgenus *Eu-Sorghum*. The innumerable varieties and forms met with in the species of this sub-genus constitute a wealth of still unexplored variation. A detailed karyomorphological study of the species belonging to this group is being undertaken since such a knowledge can confidently be expected to aid considerably in the better understanding of the genetics, taxonomy and phylogenetic evolution of the grain Sorghums.

The mitotic chromosomes of *S. anakolib* can be classified into six types on the basis of relative length, position of centromere and absence or presence of secondary constrictions at metaphase. But it is not possible, on the basis of these criteria alone, to identify each of the ten chromosomes present in the haploid complement. For instance, 5 out of the 6 chromosomes falling in the " short " class cannot be differentiated from each other in the absence of any further visible morphological differences, though the sixth can be picked out because of its great asymmetry.

The pioneering and careful work of McClintock (1929) has shown that a study of pachytene chromosomes can furnish additional criteria. In general, two types of chromosomes have been found at pachytene: those which exhibit considerable morphological differentiation, and hence are easily identified as in *Zea mays* (Longley, 1941), and those which are more un-differentiated, as in *Secale* and *Agapanthus* (Lima-de-Faria, 1952). The present type of pachytene chromosome stands in between these two extremities. The main difference between the chromosomes of *Plantago ovata*, *Eu-Sorghums*, and tomato and that of *Secale*, lies in the distribution of heterochromatin material. In the former plants, the heterochromatin material is mostly noticed in the proximal ends while in the later plants heterochromatin is distributed all along the chromosome giving the appearance of a beaded or chromomeric pattern. Because of this differential distribution of heterochromatin material the former type of chromosomes, wherein the heterochromatin material appear to be concentrated at the proximal ends, look like prochromosomes in the early stages of mitotic division unlike in the later case where the heterochromatin material is distributed all along the length of the chromosome.

The pachytene chromosomes of *Eu-Sorghums* are characterized by striking differential stainability and distinct centromeres while in the *Para-sorghums* they are uniformly stained with no marked accumulation of stain in any one region of the chromosome. Thus, Garber (1948) described the pachytene chromosomes in *S. intrans*, a *Para-sorghum*, as staining uniformly. Reddi (1958) found that in the pachytene chromosomes of *S. purpureo-sericeum*, another *Para-sorghum*, the chromomeres showed a close gradient in size from the proximal to the distal end. On the other hand as stated above, all *Eu-Sorghums* thus far studied show prominent differentially staining regions. Garber (1950) studied the pachytene chromosomes of *Sorghum vulgare* and found regions immediately adjacent to the centromeres to be heavily stained, the quality of staining decreasing noticeably beyond these regions with distal parts of the chromosome being unstained. Venkateswaralu and Reddi (1956) described the pachytene chromosomes in *S. subglabrascens* and also observed the differential stainability of the pachytene chromosomes in this species. A similar differentiation into deeply stained proximal and unstained distal parts has been noted in the present case also. It appears, therefore, rather unlikely that the *Para-sorghums* have played any great role in evolution of the *Eu-Sorghums*. The results of the present study show some similarities with the pachytene chromosomes of *S. subglabrascens* as regards the relative length and position of centromeres. But some differences have also been noticed concerning the chromomeric pattern and arm ratio, particularly of the VI chromosome. Thus Venkateswaralu and Reddi (1956) report an arm ratio sometimes exceeding 1:5 for the VI chromosome in *S. subglabrascens*, but in the species under


Fig. 3. Camera lucida diagram of the somatic chromosomes from root tip squash. (X1500).
Fig. 4–6. Early diakinesis, late diakinesis and Metaphase I, showing chiasmata. (X1800, X1800 and X1500 respectively).
Fig. 7. Anaphase I showing late separation of one bivalent. (X1500).

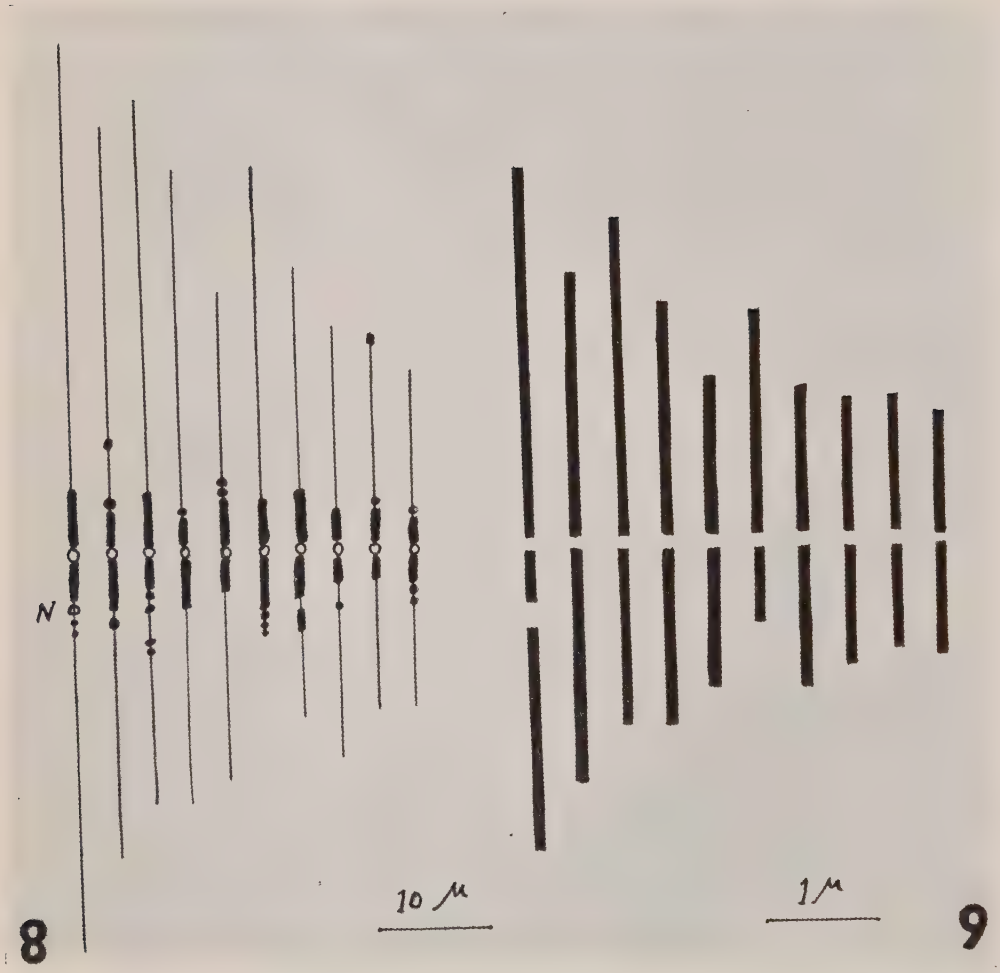


Fig. 8. Idiogram of the pachytene chromosomes showing the details of their morphology (N–Nucleolus organizer).
Fig. 9. Idiogram of the somatic chromosomes.

investigation it was always less than 1:5. Differences in chromomeric pattern also appear to be present.

Pairing between homologous chromosomes as also their separation may be initiated near the centromeric region or it may start at the distal ends and proceed towards the centromere. Thus, Brown (1949), in tomato, found that pairing is initiated during zygotene or early pachytene stage in the distal achromatic zones followed later by pairing in the chromatic zones. Hyde (1953), in *Plantago ovata*, found that pairing is initiated regularly in the middle segments while separation of the divided sister chromosomes appeared first in the end segments during the early diplotene stage. Darlington (1933a and b, 35) showed that in *Fritillaria* and *Agapanthus* initial pairing as well as diplotene separation takes place in the condensed proximal regions. In the present material the initiation of pairing is in line with that seen in *Fritillaria* and *Agapanthus* (see Darlington 1937) but the separation of the split chromosomes starts at the distal end as in *Plantago ovata*. Incidentally, this latter observation is in contrast to the findings of Venkateswaralu and Reddi (1956) in *S. subglabrascens*.

Brown (1949) and Barton (1950) reported localization of chiasmata in the achromatic region in tomato. Venkateswaralu and Reddi (1956) have, on the basis of the assumption that chiasmata are randomly distributed over the entire chromosome complement, suggested that in the short arm of Chromosome VI, which is largely constituted of deeply staining regions, a great proportion of the chiasmata should be occurring in the proximal darkly staining region. This statement has not been supported by cytological evidence, nor does it take into account the possibility of genetically determined localization of chiasmata to the ends of the chromosomes.

However, in the present study, microscopic observation during diakinesis has shown that chiasmata are very seldom formed in the chromatic region. If there is such a localization, it would mean, recombination of the genes located on the heavily stained region would be highly restricted.

A comparative study between the somatic chromosomes and pachytene chromosomes, considering only the main morphological features such as relative length and the position of the centromere (Table 2), shows a close agreement. However, chromosome VI, the most asymmetrical one in the complement has an arm ratio of 1:5 in pachytene but shows an arm ratio of 1:3 or 1:2·5 at somatic metaphase and occupies the 5th, and sometimes 6th, place. However, on the basis of a careful study of different stages of pachytene, the authors have placed this chromosome in the 6th place in the mitotic idiogram also.

A close observation was made, using the 6th chromosome because of its easy identification due to asymmetry and shortness in the complement, on the process of condensation of the chromosomes. The arm ratio of this chromosome was found to be modified from 1:5 in the mid or early mid-pachytene to 1:3 or 1:2·5 in late pachytene. In as much as the absolute length of the short arm remained almost constant at all stages of pachytene, it may be inferred that the variability in the arm ratio of this chromosome is due to the change in the length of the long arm consequent on spiralization.

It may be pointed out here that there are some apparent drawbacks in pachytene analysis. An extensive study of pachytene chromosomes in this species showed that relative lengths, arm ratio and number of chromomeres are very variable and thus render the absolute identification of any isolated chromosome very difficult at times. Gottschalk (1954) has demonstrated in *tomato* that much of this variation is independent of the stage examined. On the other hand, variation observed in the present material, particularly in the VI chromosome appears to be highly correlated with the stage and thus, classification of this chromosome on the basis of chromosome length or arm ratio may vary with the stage studied, especially as spiralization also appears to be autonomously controlled to some extent by each individual chromosome. Other

criteria used at this stage, such as chromomere pattern or the number of macrochromomeres visible at this stage may also be influenced by external factors and thus prove to be of restricted reliability (Magoon and Ramanujam, 1960).


## Summary

Karyomorphology of *S. ankolib* var. annalib red was studied both at somatic metaphase in root tip cells and at pachytene in pollen mother cells. Idiograms of the somatic chromosomes are presented on the basis of relative length and centromere position. Pachytene chromosomes are depicted on the basis of relative lengths, centromere position, arm-ratio and number of chromomeres which are nearer to the darkly staining region. A close agreement between the two idiograms was noticed with regard to major factors like relative length, arm-ratio and position of the centromere. Both pachytene chromosomes and somatic chromosomes exhibit differential staining regions which persist till late diakinesis.

Studies on the pairing properties of the differentially stained regions show proximally that synapsis starts while separation of the split chromosomes starts from distal end. The distribution of chiasmata at different stages and the terminalization co-efficient was studied. A critical analysis of the bivalents at diakinesis revealed that chiasmata are absent or rarely present in the deeply staining region. If there is such a localization, it would mean recombination of the genes located in the heavily stained region would be highly restricted.



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# GENETICAL STUDIES ON THE EFFECT OF X-RAYS IN THE SILKWORM (*BOMBYX MORI* L.) III. ON THE DIFFERENCE IN THE X-RAY INDUCED DEFICIENCIES AT **pe** AND **re** LOCI IN VARIOUS STRAINS

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Herskowitz and Abrahamson (1956) demonstrated that radiation doses upto 2500 r produced no observable reduction in the number of eggs laid by *Drosophila* females. They also noticed that the relatively higher frequency of pseudo-crossovers plus crossovers, as compared to gross structural changes involving the same region, in late oocytes was due to recombination rather than to restitution. In the silkworm, Nakao (1953) showed that the magnitude of difference in the mutation rate at specific loci between treated and non-treated moths depended upon the X-ray dose and that, with almost equal X-ray doses, spermatozoa entering four hours after exposure of moths to irradiation gave a lower mutation frequency than those entering two and a half hours after treatment. Tajima (1957) irradiated female and male germ cells with beta-rays at different larval stages and concluded that spermatogonia and spermatocytes were very sensitive to radiation, while spermatids and spermatozoa were fairly resistant.

The present experiment was designed to test the increase in mutation rate (from uncovering at **pe** and **re** loci) by irradiating male and female pupae (one day before moth emergence) of several normal ( + / + ) strains and by crossing these with **pe re**/**pe re** females and males.

## Materials and Methods

Pure line progenies of several normal and mutant strains of different races reared in the spring of 1958 were utilised. The seven strains investigated were $N_1$ (Japanese race, univoltine), Cambodge (Indian race, multivoltine), Daizo (Chinese race, bivoltine), Mus-1 (Chinese race, bivoltine), C4 (Chinese race, univoltine), C 106 and C 108 (both Chinese races, bivoltine), and homozygous **pe re** individuals that laid white eggs. Both **pe** and **re** genes are located on chromosome V, at 0·0 and 31·7 units respectively. Normal colour of the eggs (+ + / **pe re**) is purple, **pe**/**pe** (as well as **pe re**/**pe re**) eggs are white, while **re**/**re** eggs are red.

Rearing of the silkworm was done according to the method popularly known as Paraffin Paper Method. The fundamental principle of soft-leaf feeding in early and ordinary leaf feeding in advanced larval stages was strictly observed. Six days after moulting, cocoons were cut open at one end for correct positioning of pupae inside, and kept at room temperature throughout.

The experiment was divided into two parts. In the first part, female pupae of the normal strains were irradiated and the moths emerging from the pupae were mated with **pe re**/ **pe re** males. In the second, male pupae of all the normal strains were irradiated and the males emerging from these were mated with **pe re**/**pe re** females. Individuals which emerged more than one day after the X-ray treatment were rejected.

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Eggs obtained from female moths were kept at room temperature (22° C) for about six days, after which scoring of the total, unfertilized and dead, and mutant eggs was done.

A uniform dosage of 4000 r was administered at 180 kV., 25 mA. with 1 mm. Al filter at a distance of 18 cm. from the source, delivered at the rate of 1210 r per minute. The irradiations were carried out at room temperature (21° C).

## Results and Discussion

Controls have been omitted because experience with various strains showed that the occurrence of spontaneous mutation at the **pe** and **re** loci is either absent or very low and hence negligible.

*Experiment I.*—Results summarised in Table 1 show that uncovering at the **pe** locus is the highest in the strain C 4, and almost equal in Daizo, the values for these two strains being highly significant over those for N1, C 108 and C 106. The latter three strains give almost equal uncovering precentage at **pe** locus. C 4 also leads with a (statistically significant) value of 7·97 per cent uncovering at **re** locus followed by C 108, N 1, Daizo, and C 106 with 2·0, 1·94, 1·0 and 0·33 per cent uncoverings respectively.

### Table 1

*Frequency of mutations obtained by x-irradiating female pupae of several strains (one day before moth emergence) and mating them with* **pe re**/**pe re** *males* (4000 *r*)

| Cross | Total No. of eggs | Unfertilised and dead eggs (%) | No. of eggs tested | No. of White eggs (**pe**) | No. of red eggs (**re**) | Mosaics | | | % of Uncovering | | Total Mutation % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | +/**pe** | +/**re** | **pe**/**re** | **pe** locus | **re** locus | |
| N1×**pe re** .. | 1076 | 8·92 | 979 | 20 | 15 | 3 (0·3%) | .. | 4 (0·4%) | 2·75 | 1·94 | 4·69 |
| Daizo×**pe re** | 1406 | 8·03 | 1293 | 65 | 9 | 11 (0·85%) | .. | 4 (0·31%) | 6·19 | 1·0 | 7·19 |
| Mus-1×**pe re** | 483 | 100·0 | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| C4×**pe re** .. | 695 | 15·25 | 589 | 34 | 38 | 5 (0·85%) | 5 (0·85%) | 4 (0·6%) | 7·13 | 7·97 | 15·10 |
| C108×**pe re** | 584 | 48·8 | 299 | 7 | 6 | .. | .. | .. | 2·34 | 2·0 | 4·34 |
| C106× **pe re** | 357 | 15·96 | 300 | 6 | 1 | .. | .. | .. | 2·0 | 0·33 | 2·33 |

During the course of these observations, three types of egg mosaics, phenotypically +/**pe,** +/**re** and **pe**/**re,** were met with. +/**pe** mosaics appeared in N 1, Daizo and C 4 with a frequency of 0·3, 0·85 and 0·85 per cent respectively. The +/**re** mosaic was not observed in any of the strains other than C 4 (0·85 per cent). **pe**/**re** mosaics occurred only in the strains that gave rise to +/**pe** mosaics. It seems, therefore, that the occurrence of these two types of mosaics is correlated.

Perusal of the data given in Table 1 reveals that total mutation rate at **pe** and **re** loci is the highest in the strain C 4, with 15·1 per cent uncovering, which is highly significant over the rate in N 1, C 108 and C 106. The mutation rate in the other

four strains studied was 7·19, 4·69, 4·34 and 2·33 per cent for Daizo, N 1, C 108 and C 106 respectively. The mutation rate for Daizo was statistically highly significant over those of the other three. Chi-square analysis according to Snedecor's method showed that the probability of mutation is highly dependent, with $P<0·01$, upon the female parent.

The rate of mutation at **pe** and **re** loci apparently seems to have no relation with the induction of egg lethality (Tables 1 and 2).

*Experiment II.*—In Table 2 as well, the inter-varietal difference in the total mutation frequency, both at the **pe** and the **re** locus is highly dependent upon the type of the cross ($P<0·01$). Uncovering of the **pe** locus took place in all the crosses; the highest being 5·4 per cent noted in **pe re** X Mus-1, and 4·2 per cent in **pe re** X C 106, both being significantly greater than that in all other crosses except **pe re** X Daizo. The last case is statistically significant over **pe re** X N1 and the other two crosses. ($P<0·01$)

TABLE 2

*Frequency of mutations obtained by x-irradiating male pupae of several strains (one day before moth emergence) and mating them with* **pe re**/**pe re** *females* (4000 *r*)

| Cross | Total No. of eggs | Unfertilized & dead eggs % | No. of eggs tested | No. of white Eggs (**pe**) | No. of red eggs (**re**) | Mosaics +/**pe** | % of Uncovering | | Total Mutation % |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | **pe** Locus | **re** Locus | |
| **pe re** × N1 | 1102 | 11·79 | 972 | 3 | 0 | 1 | 0·41 | 0·0 | 0·41 |
| **pe re** × Cambodge | 801 | 1·12 | 792 | 2 | 1 | 1 | 0·39 | 0·12 | 0·51 |
| **pe re** × Daizo | 332 | 2·4 | 324 | 3 | 3 | 1 | 1·2 | 0·9 | 2·1 |
| **pe re** × Mus-1 | 1009 | 5·45 | 946 | 51 | 0 | 0 | 5·4 | 0·0 | 5·4 |
| **pe re** × C 4 | 999 | 1·9 | 980 | 6 | 0 | 2 | 0·82 | 0·0 | 0·82 |
| **pe re** × C 108 | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| **pe re** × C 106 | 187 | 23·53 | 143 | 6 | 0 | 0 | 4·2 | 0·0 | 4·2 |

Mutation at **re** locus occurred only in Cambodge and Daizo with the very low, though quite different, frequencies of 0·12 and 0·9 per cent respectively. Uncovering at **re** locus in Daizo is almost 8 times that in Cambodge.

X-irradiated female pupae, as already mentioned, yielded three types of egg mosaics, whereas when the male pupae were irradiated only +/**pe** mosaics occurred. This, taken together with other observations reported above, shows that **re** locus is less mutable than **pe** locus in the males.

Further, chi-square analysis, proves that differences in the total mutation rate in tables 1 and 2 are highly sex-dependent. Taking into consideration various strains sex-wise, it would be found that differences in the frequency of mutation between reciprocal crosses of N 1 and **pe re,** Daizo and **pe re,** and C4 and **pe re** are statistically highly significant, which suggests the sensitivity of oocytes is very high, in terms of mutation frequency, as compared to that of the sperm.

The mutant eggs which revealed themselves as a result of uncoverings at **pe** and **re** loci may be regarded as the consequence of point mutations, small deficiencies or other gross structural changes (Tajima, 1957). It is difficult to identify the type of

mutation induced by X-rays and reported here, but it can now be said with a fair degree of confidence that treatment of oocytes yields far higher mutation rates than irradiation of sperms. However, Tajima and Onimaru (1958), in a paper published while this article was under preparation, have reported that there is no difference in the proportion of fertilised and early dying eggs, irrespective of whether the male or female was irradiated in the late pupal stage. This report, therefore, does not agree with the results presented here. They, however, agree on two points:—(i) mutations at **pe** and **re** loci are not necessarily related to unfertilised and early dying eggs, and (ii) oocytes are far more sensitive to X-rays than are sperms.

It may be of interest to note here that in *Drosophila*, females yield fewer mutations than males when subjected to radiation treatments (Patterson and Muller, 1930; Shapiro and Neuhaus, 1933; Oliver, 1935; Glass, 1955), and that sperms X-rayed within females give significantly higher mutation frequency than those irradiated in males (Abrahamson and Telfer, 1954; Lüning, 1953, 1954; Bonnier and Lüning, 1953).

In general, two hypotheses have been advanced to explain this difference. Patterson and Muller (1930) suggested that destruction of the gene and loss of its power to multiply bring about chromosomal aberrations, and restitution or other arrangement may follow (*see also* Glass, 1955a). On the other hand, Sidoroff (1931) and Timoféff-Ressovsky (1934) preferred germinal selection as an explanation. Glass (1955) remarked, from his findings, that reunion between broken ends decreases more rapidly with the distance between breaks in oocytes than in spermatozoa. It is for future work to decide as to which of these two hypotheses is applicable to the results presented here.

Tajima (1947) attributes the origin and production of mosaic eggs in silkworm to the occurrence of deficiencies at the marked loci. In this case mutations at **pe** and **re** loci would take place immediately during or after first cleavage division after fertilisation, whether the male or female is subjected to irradiation, since any mutation at the **pe** or **re** locus before fertilisation should, as a rule, result in non-mosaic, mutated eggs. This, however, does not imply that the phenomenon of mosaicism is solely restricted to cleavage division; in such a case, of course, the mosaics would present an irregular outline. Irregular mosaics might also appear due to irregular migration and deposition of cleavage nuclei. The origin of mosaics can be mainly considered to be due to double fertilisation, or abnormal fertilisation between egg nucleus and a polar body (Nakao, 1953). +/**pe** mosaics can also be thought of as being due to dispermic merogony. The case of **pe**/**re** presents an interesting problem since such a mutation has been found for the first time and even that only when females are irradiated. The explanation of such a type of mosaic requires a deficiency at **pe** locus and a mutation at **re** locus. From the data obtained in the present experiment it is difficult to assess the relative contribution of various factors to the formation of egg mosaics.

Since the treatment of X-radiation was given in all cases to mature sperm and oocyte, the possibility of uncoverings at **pe** and **re** loci due to gross chromosomal changes is rather remote. Most of these mutations, therefore, might be the result of point mutation or deficiencies. However, it is easy to reconcile the results of the **re** locus with point mutations rather than deficiencies because **re** is located at 31·7 map units on the Chromosome V. On the contrary, **pe** is located at one end of chromosome V (0·0), and consequently the high mutation frequency obtained with regard to this locus may be attributed to deficiency and to a lesser extent to point mutations. This aspect of the problem needs further attention.

## Summary

Data have been collected which yield information on the frequency of mutations at **pe** and **re** loci (Chromosome V) in the silkworm. In one part of the study, female

pupae of normal strains were subjected to a dose of 4000 r of X-rays, one day before moth emergence, and then mated on emergence with **pe re** males. Strains of races N 1, Daizo, Mus-1, C 4, C 108 and C 106 were used.

It was found that intercross difference in unfertilised and dead eggs, and uncoverings of **pe** and **re** loci were extremely dependent upon strains ($P < 0{\cdot}01$).

Mutation frequencies as a result of uncovering at **pe** and **re** loci were 15·1, 7·19, 4·69, 4·34, and 2·33 per cent respectively in C 4, Daizo, N 1, C 108 and C 106. Most of the crosses showed very highly significant differences.

In the second part of the investigation, male pupae were irradiated with 4000 r of X-rays one day before emergence and were then mated on emergence to **pe re** females.

Observations made on the hybrid eggs revealed that the difference in unfertilised and dead eggs, and mutation percentage (for **pe** and **re** loci) are highly strain-dependent ($P < 0{\cdot}01$).

Mus-1, C 106, Daizo, C 4, Cambodge, and N1 gave values of 5·4, 4·2, 2·1, 0·82, 0·51 and 0·41 respectively in the form of uncoverings at **pe** and **re** loci. Sixty per cent of the six crosses gave differences that were statistically highly significant.

X-irradiation of oocytes gave higher uncoverings at **pe** and **re** loci than of sperm which indicates that the latter are more resistant to X-rays than the former.

**re** locus is less mutable than **pe** locus. It is probable that this difference could be due to the greater frequency of deficiencies at the **pe** locus which is located near one end of Chromosome V than in the interstially located **re** locus.

Two types of mosaics, regular and irregular, were met with in this experiment. Phenotypically, three types were found viz., +/**pe**, +/**re** and **pe**/**re** mosaics in the case of irradiated females while only one type viz., +/**pe** was found in the case of irradiated males.

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# INHERITANCE STUDIES IN WHEAT—VII. INHERITANCE OF SEEDLING REACTION TO PHYSIOLOGIC RACES 24 AND 117 OF *PUCCINIA GRAMINIS TRITICI* IN SOME INTERVARIETAL CROSSES OF COMMON BREAD WHEAT

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Observations on the inheritance of seedling reaction to races 13 and H of stripe rust, *Puccinia glumarum* (Schm.) Erikss and Henn., have been reported by Bahl and Kohli (1960). Results of similar studies conducted with races 40, 42, 75 and biotype 42-B of stem rust, *Puccinia graminis tritici* Erikss. and Henn. have been reported earlier (Rao and Agrawal, 1960). It was found that reaction to these forms of stem rust is relatively simply inherited, being governed by either one or two gene pairs. These studies were extended to other Indian forms of stem rust and in the present paper observations on the mode of inheritance of resistance to physiologic races 24 and 117 are reported. There does not appear to be any report on the inheritance of seedling reaction to these races of stem-rust.

These studies have been carried out in crosses involving varieties of the common bread wheat, *Triticum aestivum* L. The resistant wheats involved are N.P. 790, (Kenya-Gular-Pilot) × K58-New Thatch (E.1913), Ridley (E. 572) and (Timstein × 2086) Sel. 1495-A-1-7-3-1 (E. 871), while the susceptible wheats are N.P. 165, N.P. 710, N.P. 718 and N.P. 775.

## Materials and Methods

The material under study comprised of the following five crosses. Races against which the material has been tested are also indicated.

| Sl. No. | Cross | Material tested | Races used |
|---|---|---|---|
| 1. | N.P. 710 (S) × E. 1913 (R) | Parents, $F_1$ and $F_2$ | 24 and 117 |
| 2. | N.P. 710 (S) × N.P. 790 (R) | Parents and $F_2$ | 24 |
| 3. | N.P. 775 (S) × N.P. 790 (R) | Parents, $F_1$ and $F_2$ | 24 and 117 |
| 4. | E. 871 (R) × N.P. 165 (S) | Parents, $F_1$ and $F_2$ | 24 and 117 |
| 5. | N.P. 718 (S) × E. 572 (R) | Parents and $F_2$ | 24 and 117 |

Varieties N.P. 710, N.P. 718, N.P. 775 and N.P. 165 are susceptible in the seedling stage to all the races of black rust so far reported from India. N.P. 790 which was derived from the cross Thatcher × N.P. 165 is a very valuable source of black rust resistance in India. It is resistant to all the races and biotypes of this rust recorded in India except 122. Ridley at one time was used as a resistant stock for black rust, but subsequently it was found susceptible to 122 and biotype 15-C. Further, it is now known that its resistance to some of the other races breaks down at higher temperatures.

*Puccinia graminis tritici*, race 24 was isolated by Mehta from the collections made from the crop of 1932-33. The race is not very widely distributed and is of moderate virulence. Race 117 was first isolated from the crop of 1944-45. It is a rare race of moderate virulence.

## Method

The usual standard techniques of inoculation was followed and reactions were recorded according to the system drawn up by Stakman, Levine and Loegering (1944). Plants showing 0, 1 and 2 reactions were put under resistant class while those showing 3, 4 and mesothetic reactions were considered to be susceptible.

## Results

(i) *Inheritance of seedling reaction to race* 24:

The inheritance of seedling reaction to race 24 was studied in five crosses. Data recorded on this material are summarised in Table 1.

### Table 1

*Mode of segregation of seedling reaction to race* 24 *in the* $F_2$ *generation of intervarietal crosses of* T. aestivum

| Sl. No. | Cross | Number of seedlings | | Total | $X^2$ | P Value | Mode of segregation |
|---|---|---|---|---|---|---|---|
| | | Resistant | Susceptible | | | | |
| 1. | N.P. 710(S) × E. 1913(R) | 310 | 4 | 314 | 0·1686 | 0·80 to 0·70 | 63R:1S |
| 2. | N.P. 710(S) × N.P. 790(R) | 248 | 11 | 259 | 2·481 | 0·20 to 0·10 | 15R:1S |
| 3. | N.P. 718 (S) × E. 572(R) | 279 | 24 | 303 | 1·442 | 0·30 to 0·20 | 15R:1S |
| 4. | N.P. 775(S) × N.P. 790(R) | 234 | 22 | 256 | 2·40 | 0·20 to 0·10 | 15R:1S |
| 5. | E. 871(R) × N.P. 165(S) | 268 | 76 | 344 | 1·552 | 0·30 to 0·20 | 3R:1S |

The resistant varieties, E. 1913, N.P. 790, E. 572 and E. 871 showed reactions ranging from 0 to 1 while N.P. 710, N.P. 718, N.P. 775 and N.P. 165 were all susceptible, showing type 4 reaction. $F_1$, which was tested only in three crosses, showed dominance of resistance.

The $F_2$ data showed, that E. 1913 carried three dominant duplicate genes for resistance to race 24, while N.P. 790 and E. 572 carried two duplicate dominant genes. E. 871 was observed to carry only one dominant gene for resistance.

(ii) *Inheritance of seedling reaction to race* 117:

Four crosses were studied for finding out the mode of inheritance of seedling reaction to race 117. Data recorded in the $F_2$ generation of these crosses are presented in Table 2.

TABLE 2

*Segregation for seedling reaction to race* 117 *in the* $F_2$ *generation of four intervarietal crosses of* T. aestivum

| Sl. No. | Cross | Number of seedlings | | Total | $X^2$ | P Value | Mode of segregation |
|---|---|---|---|---|---|---|---|
| | | Resistant | Susceptible | | | | |
| 1. | N.P. 710(S) × E. 1913(R) | 312 | 8 | 320 | 1·828 | 0·20 to 0·10 | 63R:1S |
| 2. | N.P. 775(S) × N.P. 790(R) | 344 | 30 | 374 | 1·985 | 0·20 to 0·10 | 15R:1S |
| 3. | N.P. 718(S) × E. 572(R) | 310 | 26 | 336 | 1·269 | 0·30 to 0·20 | 15R:1S |
| 4. | E. 871(R) × N.P. 165(S) | 239 | 65 | 304 | 2·751 | 0·20 to 0·10 | 3R:1S |

The varieties N.P. 790, E. 1913, E. 871 and E. 572 showed reactions ranging from 0 to 1 while the reaction type varied from 3 to 4 in the susceptible varieties N.P. 710, N.P. 718, N.P. 775 and N.P. 165. All the $F_1$ plants, in those crosses for which seed was available for testing, were resistant thereby showing dominance of resistance.

The $F_2$ data indicated that resistance of E. 1913 to race 117 is governed by 3 pairs of duplicate dominant genes while that of E. 572 and N.P. 790 depends on two such pairs. Monogenic mode of inheritance, resistance being dominant, has been observed in the cross E. 871(R) × N.P. 165(S).

## DISCUSSION

The observations described above suggest that the genetic determination of resistance in relation to both these races is of a simple nature. This, as well as the fact that resistance shows complete dominance over susceptibility, makes it clear that breeding for resistance to these races, making use of the resistant parents involved in these crosses, should present no serious difficulty.

Makhija (unpublished, 1958) observed resistance of N.P. 790 to race 21 of stem rust to be governed by two duplicate dominant factors in crosses with N.P. 710 and N.P. 775 whereas he observed a unifactorial segregation, with dominance of resistance, in the cross E. 871(R) × N.P. 718(S).

From these results, it appears that the mode of inheritance in E. 1913 (Kenya-Gular-Pilot × K. 58-New Thatch) for resistance to both 24 and 117 is similar. Similarly, the mode of inheritance in N.P. 790 and E. 871 (Timstein × 2086 Sel. 1495A-1-7-3-1) appears to be the same, with regard to resistance to races 24, 117 and 21. Whether the genes governing resistance to these different races are the same or different, needs further study.

## Summary

Results of studies conducted with a view to finding out the number of genes involved in controlling seedling resistance of N.P. 790, E. 1913, E. 572 (Ridley) and E. 871 to races 24 and 117 of stem rust are reported.

Resistance of N.P. 790 and E. 572 to races 24 and 117 was observed to be governed by two pairs of dominant duplicate genes.

Three pairs of duplicate dominant genes were found to determine the resistance of E. 1913 to races 24 and 117.

E. 871 (Timstein × 2086 Sel. 1495A-1-7-3-1) was found to carry one pair of dominant factors for resistance to races 24 and 117.

Further studies are needed to show whether the genes governing resistance to the two races are the same or different.



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# STUDIES ON THE CHEMICAL INDUCTION OF POLLEN STERILITY IN SOME CROP PLANTS

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Exploitation of hybrid vigour has now become a potent tool in plant breeding. The successful utilization of this phenomenon in enhancing crop production, however, depends on the economics of hybrid seed production. In crops which are not as fortunately placed as maize with regard to the possibility of producing hybrid seeds cheaply and on a large scale, extensive research is in progress to isolate spontaneously occurring male sterile lines. Attempts have also been made to induce the selective abortion of pollen grains through treatment with various chemicals (see review by Jain, 1959). Since chemical induction of pollen sterility appears to offer some promise in several crop plants, experiments were started at the Indian Agricultural Research Institute in 1955, to standardise suitable techniques for causing male sterility in wheat, tomato and onion. The results of this study are reported in this paper.

## Materials and Methods

Wheat (variety C. 591), tomato (variety Sioux) and onion (variety Pusa Red) were taken up for induction of pollen sterility. The stage of treatment, the concentrations at which various chemicals were used and the method of treatment are described below for each crop.

*Wheat Maleic hydrazide* (*MH*), was used at 50, 100, 250 and 500 parts per million Thirtyseven-day old plants were first treated bys praying aqueous solution of the chemical. Successive sets were treated at 15 day intervals before the emergence of flag leaf (3 sets). A fourth set was treated when the flag leaf had fully appeared. Spraying was repeated on consecutive days within each set, one to four times. Maleic hydrazide was obtained from the U.S. Rubber Company, as MH 30 (formulation containing 30 per cent active ingredient.)

*Gametocide, F.W.* 450, chemically sodium $\alpha$, $\beta$, dichloroisobutyrate, was obtained from Rohm and Hass company, U.S.A., as a 100 per cent active, water soluble salt. Wheat plants were sprayed once or twice with 0·25, 0·5, 0·75 and 1·0 per cent aqueous solution of this chemical.

*Tomato.*—Tomato plants were sprayed once or twice with aqueous solutions of different chemicals about a week prior to the opening of the first flower. The concentrations at which the chemicals were used are given below:

| Chemical | Concentrations |
|---|---|
| MH, Uracil and Thymine | 50, 100, 250 and 500 ppm. |
| Yeast Nucleic acid | 0·1, 0·25 and 1 per cent. |
| Tri-iodobenzoic acid (TIBA) | 10, 50, 100 and 250 ppm. |

In the case of maleic hydrazide, the treatments were repeated during the second flush before flower opening, after removing the flowers of the first flush, to find out whether the concentrations which brought about complete pollen abortion at an early stage of growth, worked well at a later stage also.

*Onion.*—Aqueous solutions of six chemicals listed below were injected into the inflorescence-bearing stalk prior to the onset of meiosis in the oldest bud of the inflorescence.

| Chemical | Concentrations tried |
|---|---|
| Maleic hydrazide | 10, 50, and 100 ppm. |
| Uracil | 50, 100, 250 and 500 ppm. |
| Thymine | 50, 100, 250 and 500 ppm. |
| Nucleic acid | 0·1, 0·25, 0·5 and 1 per cent |
| Sodium nucleate | 1, 2, 3 and 4 per cent |
| Tri-iodobenzoic acid | 10, 50 and 100 ppm. |

*Pollen Fertility.*—Pollen fertility was estimated in aceto-carmine preparations. Ten or more random fields all over the slide were counted, making a total of 100 to 200 pollen grains. Fertility counts were taken at weekly intervals starting from the first flower opening till recovery of complete fertility.

*Pollination and seed-set.*—For determining ovule fertility in chemically induced male-sterile plants, pollinations were done with pollen from male-fertile (control) plants during the period of complete male-sterility. A comparison of seed-setting in fruits resulting from hand pollination of flowers on male-sterile plants with the seed-setting in controls provided an index of the ovular fertility and seed-setting capacity of flowers on the treated plants.

## Experimental Results

### *WHEAT*

*Effect of Maleic Hydrazide:*

(a) *Meiosis.*—In plants treated with a concentration of 100 ppm. and above of MH before the emergence of the flag leaf, abnormalities such as varying number of univalents, cells with unbalanced chromosome number, stickiness at metaphase and laggards and sticky bridges at first anaphase were observed during meiosis in microsporocytes. In plants sprayed with 100 ppm. (4 times) or 250 ppm., sprayed once or twice, metaphase chromosomes were fused together into undifferentiated masses of chromatin thereby rendering a detailed study of chromosome configurations impossible. Extreme stickiness, both at metaphase and anaphase, was observed at the second division also.

(b) *Pollen fertility and seed-setting.*—The data on pollen fertility and seed setting in wheat treated with different concentrations of MH during 1955-56 are given in Table 1. It can be seen from the data that 250 ppm. and 100 ppm. were fairly effective in causing pollen sterility (see also Fig. 6). With an increase in the frequency of spraying the effect of a dosage increased. Though data on seed setting could not be obtained during 1956-57, since the crop was severely damaged by a hailstorm, plants sprayed once or twice during tillering stage with 250 ppm. MH had shrunken anthers with shrivelled pollen. Aborted anthers were also found in plants treated at the time of

emergence of flag leaf with MH concentrations of 250 ppm. sprayed two to four times and 500 ppm. sprayed once or twice.

TABLE 1

*Seed-setting in wheat treated with Maleic Hydrazide*

| Stage of treatment | Dosage | | Percentage seed-setting | |
|---|---|---|---|---|
| | Concentration in parts per million | No of applications | Selfing | Hand pollination |
| 1. Many Tiller Stage | 250 | 1 | 16·9 | 90·0 |
| | | 2 | 13·6 | 95·2 |
| | | 3 | 25·0 | 94·7 |
| | 100 | 1 | 35·7 | 100·0 |
| | | 2 | 23·3 | 86·4 |
| | | 3 | 6·4 | 100·0 |
| 2. Flag leaf emergence | 250 | 1 | 18·7 | 85·0 |
| | | 2 | 30·4 | 100·0 |
| | | 3 | 14·3 | 95·2 |
| | 100 | 1 | 74·6 | 100·0 |
| | | 2 | 80·6 | 72·7 |
| | | 3 | 81·1 | 90·0 |
| 3. Flag leaf fully emerged | 250 | 1 | 82·1 | 94·7 |
| | | 2 | 63·1 | 65·0 |
| | | 3 | 26·2 | 90·3 |
| | 100 | 1 | 81·8 | 85·0 |
| | | 2 | 67·5 | 94·4 |
| | | 3 | 31·6 | 100·0 |

(c) *Results of crossing without emasculation.*—A number of crosses were attempted during the year 1956 between the varieties C. 591 (awned) and N.P. 790 (awnless) using MH treated C. 591 as the pistillate parent, without resorting to emasculation. As awnlessness in wheat is a dominant character, it can be easily ascertained from the study of the $F_1$ whether the seeds obtained from such crosses are true hybrids.

Observations on the $F_1$ progeny raised from seeds obtained from crosses without emasculation revealed that the percentage of true hybrids was rather low in seeds from plants treated with 50 ppm., while nearly 80 per cent of those from 100 ppm. and 250 ppm. were hybrids. A very interesting situation was met with in 5 plants which showed both awned and awnless tillers in the same plant. Seeds from awned and awnless tillers were harvested separately and sown. Observation on the progenies of awned and awnless tillers showed that whereas the awned tillers bred true for the fully bearded character, the awnless ones segregated into fully awned, half bearded, long tipped, short tipped and awnless types. This clearly suggests that while fully

bearded tillers represent selfed C. 591, the awnless tillers were of hybrid origin. Data on the breeding behaviour of the progeny of awned and awnless tillers are given below.

| Progeny of | Behaviour of the progeny | | | |
|---|---|---|---|---|
| | Fully bearded | Half bearded | Tipped | Awnless |
| Awned tiller .. .. | 36 | 0 | 0 | 0 |
| Awnless tiller .. .. | 7 | 16 | 24 | 36 |

From monosomic analysis it is known that N.P. 790 has the epistatic genes $\mathbf{B_1}$ in chromosome IX and $\mathbf{b^a}$ in chromosome X (Sikka *et al.*, 1959). The gene $\mathbf{b_2^a}$ is similar to **Hd** of chromosome VIII in action and causes a reduction in the length of the awns. C-591 being fully bearded has neither $\mathbf{B_1}$ nor $\mathbf{B_2}$ or $\mathbf{b_2^a}$ and a hybrid between N.P. 790 and C-591 is, therefore, likely to give a segregation of 9:3:3:1 (awnless: tipped:half bearded:fully bearded) in the $F_2$. The origin of such chimeras is not clear—probably these plants had originated as twin embryos.

*Effect of Gametocide (F.W. 450) treatment.*—Treatment with different concentrations of gametocide F.W. 450, ranging from 0·25 to 1 per cent, caused a marked suppression of plant growth. The treated plants were very stunted and showed inward curling of leaves. About a week after treatment with 0·75 per cent and 1 per cent solutions, brown patches appeared on the leaves and the plants wilted. The plants treated with lower concentrations were dwarf and grass-like in appearance and had dark green, narrow leaves. Earing in the surviving plants was delayed by about a fortnight. The earheads were very small and had shorter awns and tougher glumes in comparison with those of the control plants. Pollen grains from the treated plants were, however, normal as regards their size, shape and stainability.

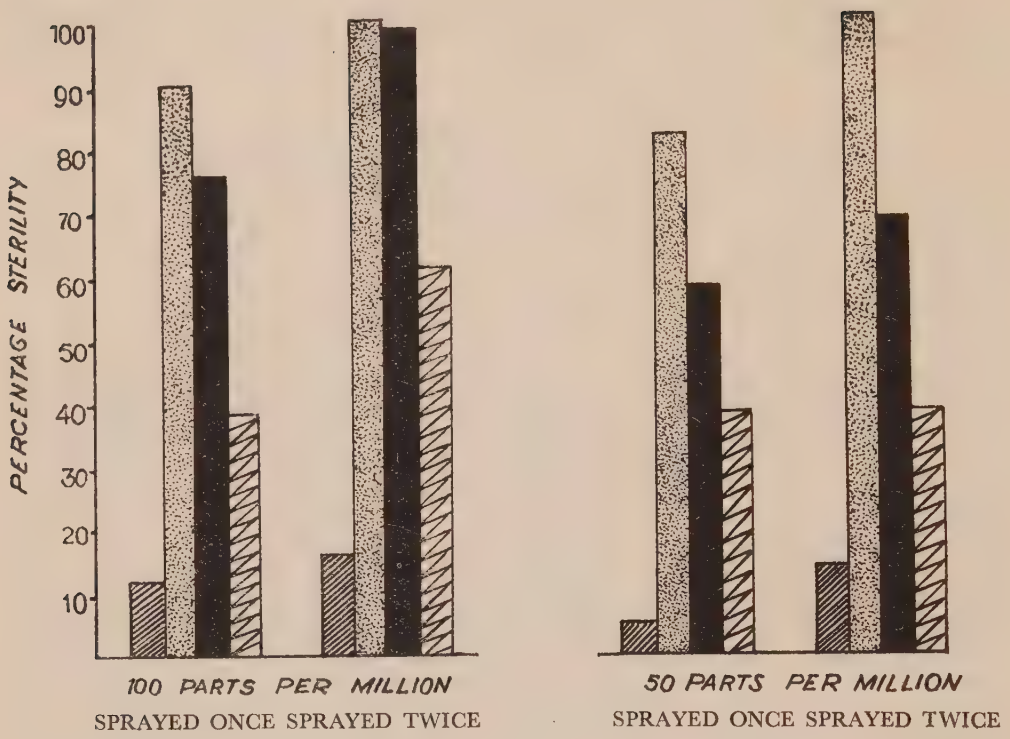
*TOMATO*

(a) *Meiosis.*—A detailed cytological study was made of plants treated with various concentrations of MH. Meiosis proceeded normally upto the tetrad stage even in plants treated with the highest concentrations. Young uninucleate microspores were shrunken and sterile (Fig. 7). The mature pollen grains at dehiscence were also deformed in shape and did not take stain in acetocarmine.

(b) *Pollen fertility and seed-setting.*—Data on pollen fertility and seed-setting are presented graphically in Text Figs. 1 and 2.

Plants treated a week before the opening of the first flower with 500 ppm. and 250 ppm., produced buds with atrophied anthers. Concentrations of 100 ppm., sprayed once or twice and 50 ppm., sprayed twice, produced complete pollen sterility. Reduction in seed setting in fruits formed during the period of complete sterility amounted to a maximum of 18·4 per cent. Pollen fertility counts at weekly intervals revealed that there was a gradual reversion to the fertile condition. Complete sterility lasted for a period of two weeks in plants treated with 100 ppm., sprayed twice and only 7 days in plants with 100 ppm., sprayed once or with 50 ppm., sprayed twice. The data from two replications were similar.

In the case of plants treated before the emergence of second flush flowers, comparatively higher concentrations were required to obtain complete pollen sterility. Thus, complete sterility associated with a reduction in seed-setting upto a maximum of 14·2 per cent, was observed in plants treated twice with 200 ppm. Here again




TEXT FIG. 1. Histogram showing effect of maleic hydrazide on pollen fertility and seed setting in tomato treated before opening of first flower. See text Fig. 2 for explanation of hatchings.

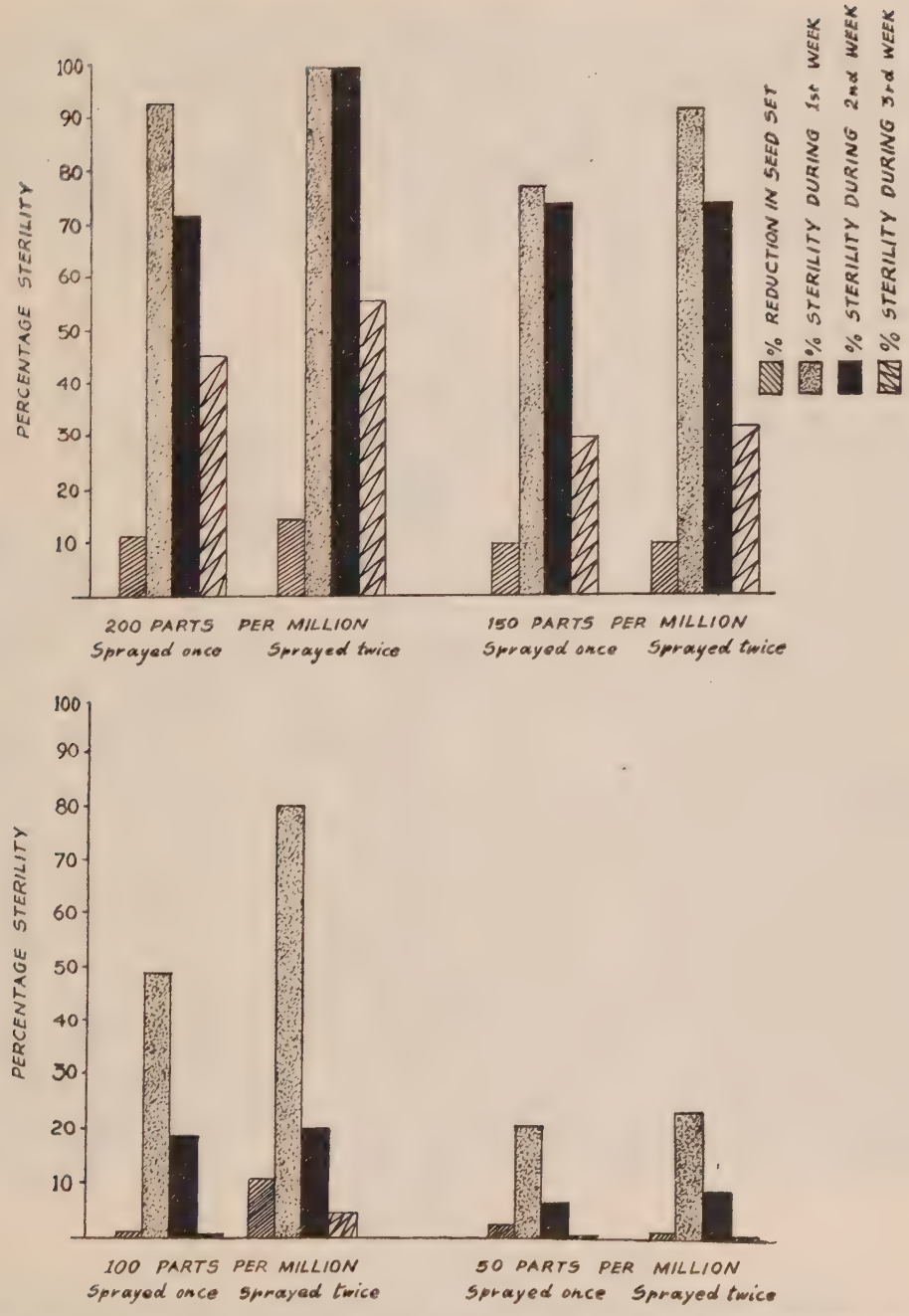
the plants recovered to a partially fertile condition after remaining completely sterile for two weeks. The treatment of two sets—one of potted plants and the other of field plants, gave identical results.

In the light of these results, it can be concluded that maleic hydrazide is capable of bringing about a selective abortion of pollen grains in tomato without reducing the seed-setting capacity to any great extent. The effective concentration, however, varies with the age of the plant; older plants require higher dosages for showing the same effects which the lower concentrations produce in plants treated at an early stage of growth.

*Other chemicals.*—Treatment with TIBA, nucleic acid, thymine and uracil, at the concentrations already stated, failed to produce abortion of pollen grains, the fertility being the same as in controls.

*ONION*

(a) *Meiosis.*—Injection of 100 ppm. MH and 1 per cent yeast nucleic acid in onion caused asynapsis and desynapsis (Figs. 1 & 2), respectively. 250 ppm. MH produced a very high degree of stickiness (Fig. 4). Treatment with 1 per cent sodium nucleate produced a series of interesting abnormalities. Normal chromosomal pairing was observed in 10·1 per cent of the cells only. Here too, a very high degree of stickiness prevented a detailed study of chromosome configurations. Polyploid cells having 32 (Fig. 3) to as many as 128 unpaired chromosomes resembling in appearance those found in the somatic tissues, formed 47·9 per cent of a total of 119




TEXT FIG. 2. Histogram showing effect of maleic hydrazide on pollen fertility and seed setting in tomato treated before flower opening in the second flush.

PMCs studied. Asynaptic cells showing 16 highly condensed unpaired chromosomes formed another 30·2 per cent of the cells. As in somatic cells, the chromosomes in some of these cells showed the two chromatids very prominently. The chromatids were found to be attached at the proximal segments of the chromosomes and not at the centric region. Thymine and uracil did not produce any abnormality in meiosis at the concentrations used.

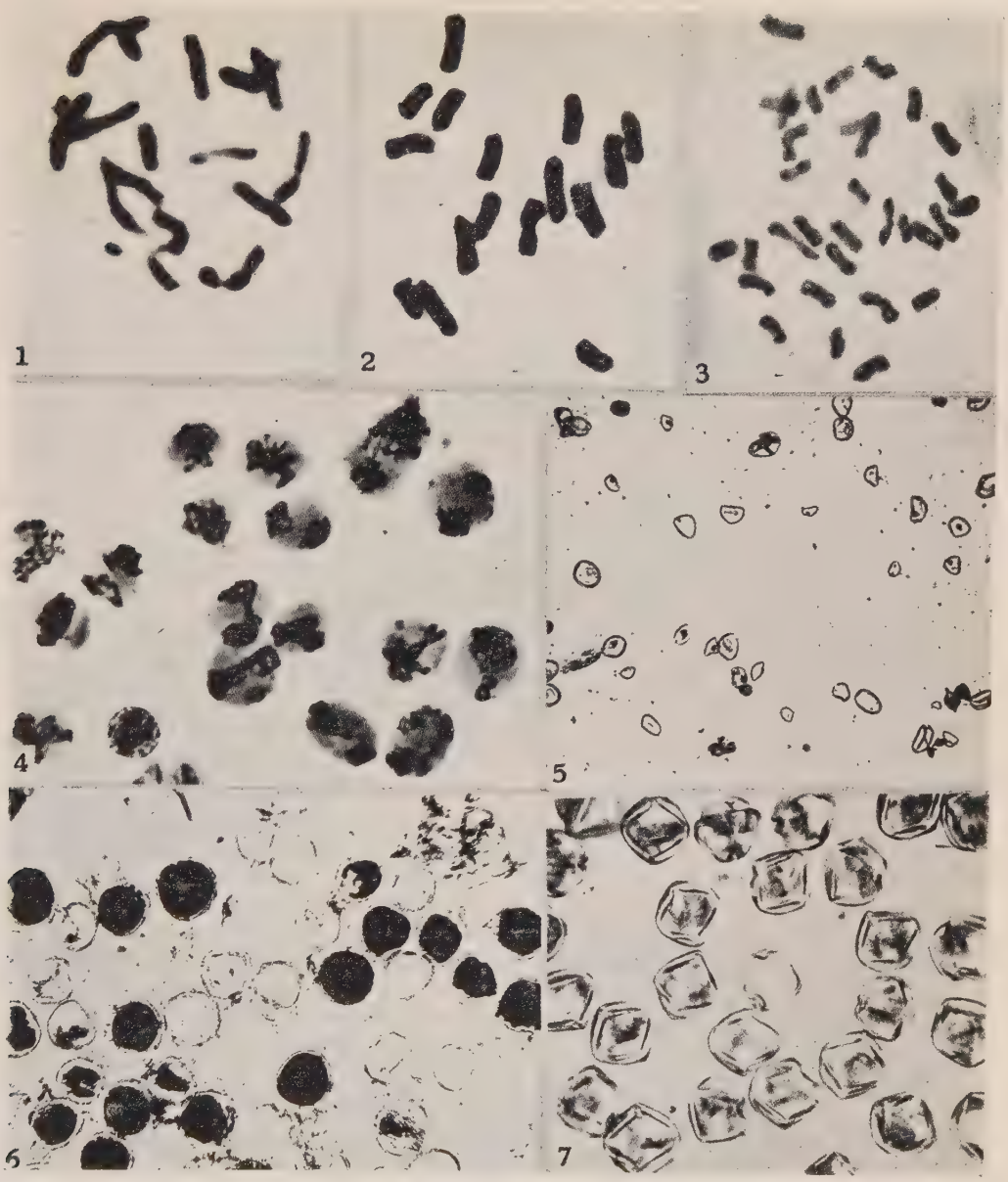
(b) *Pollen fertility and seed setting.*—The percentage of sterility produced by various concentrations of each chemical is given in Table 2 (see also Fig. 5). Unfortunately, no seed-setting data could be collected, since the crop was completely damaged by the hailstorm which occurred in March, 1957.

TABLE 2

*Effects of chemicals on pollen fertility in onion*

| Chemical | Concentration | Abnormal pollen % |
|---|---|---|
| Maleic Hydrazide .. | 10 ppm. | 100·0 |
| | 50 ppm. | 54·5 |
| | 100 ppm. | 86·7 |
| Sodium nucleate .. .. | 1 % | 78·4 |
| | 2 % | 63·4 |
| | 3 % | 98·2 |
| | 4 % | 100·0 |
| TIBA .. .. .. | 10 ppm. | 100·0 |
| | 50 ppm. | 100·0 |
| | 100 ppm. | 100·0 |
| Nucleic Acid .. .. | 0.25 % | 7·4 |
| | 0.5 % | 7·9 |
| | 1.0 % | 93·9 |
| Thymine .. .. | 50 ppm. | 28·8 |
| | 100 ppm. | 60·8 |
| | 250 ppm. | 100·0 |
| | 500 ppm. | 100·0 |
| Uracil .. .. | 50 ppm. | 47·4 |
| | 100 ppm. | 80·4 |
| | 250 ppm. | 100·0 |
| | 500 ppm. | 100·0 |

*Reproducibility of results.*—For the purpose of finding out whether comparable results are obtained during different seasons, the treatment given during 1955-56 were repeated during 1956-57. Except for slight differences in the concentrations giving the best results, the data obtained during the two years were similar.




FIGS. 1 to 4. Meiosis in pollen mother cells of onion.

FIG. 1. Diakinesis showing $16_I$. 100 ppm. MH treatment.
FIG. 2. MI with $16_I$. 1% nucleic acid treatment.
FIG. 3. A cell with 32 chromosomes. 1% sodium nucleate treatment.
FIG. 4. PMCs showing chromosome stickiness. 250 ppm. MH treatment.
FIG. 5. Sterile pollen in an onion plant treated with 4% sodium nucleate.
FIG. 6. Partially sterile pollen from a wheat plant (var. C591) treated with 100 ppm. MH.
FIG. 7. Sterile pollen grains in tomato treated with 100 ppm. MH.

## Discussion

The numerous instances of male sterility occurring among plants in nature without associated ovule sterility indicate that it may be possible to reproduce this situation artificially. Chemicals like Maleic hydrazide, Tri-iodobenzoic acid, ∝-napthalene acetic acid and 2-4, dichlorophenoxyacetic acid have been found to be useful both for inducing male sterility in several plants and for changing the ratio between male and female flowers in dioecious species (Wittwer and Hillyer, 1954; Rehm, 1952). The probable mechanism by which an auxin like NAA and an antiauxin like MH bring about the same end-result has been discussed by Jain (1959). Recently, Eaton (1957) has reported that Sodium ∝, β-dichloroisobutyrate, known commercially as FW-450, is very effective in inducing male sterility in cotton. In the case of this chemical also, the exact mechanism of action is not known. Hilton (1958) has shown that FW-450 competes with pantoate for the site on the enzyme which synthesises pantothenate. Another interesting finding obtained by using $C^{14}$ labelled FW-450 is that this chemical accumulates to a greater extent in the anthers than in the ovules. Our results, however, suggest that FW-450 is not effective as a selective "gametocide" in wheat.

Any schedule of inducing pollen sterility, to be useful in the commercial production of hybrid seeds, should fulfill the following criteria: (1) the treatment should cause only pollen abortion and not affect ovule fertility; (2) it should have no mutagenic effects; (3) the method of application should be easy and economical (4) the precise dosage when applied at a definite stage of growth in the life cycle of the plant should give consistent results i.e., the effects should be reproducible, and (5) there should be no undue hazards either to man or plant. The data available from the present study are discussed below in the light of the above requirements.

*Selective abortion of pollen.*—None of the treatments tried gave complete pollen sterility coupled with complete ovular fertility. The treatments that gave the most promising results are indicated below:

| Crop | Chemical | Concentrations giving complete pollen sterility | | % reduction in seed set | |
|---|---|---|---|---|---|
| | | 1956 | 1957 | 1956 | 1957 |
| Wheat | MH | 1. 250 ppm., once or twice during tillering stage | 1. 250 ppm., once or twice during period before flag leaf emergence | 5·3 | Data could not be collected due to hailstorm damage. |
| | | 2. 100 ppm., thrice during tillering stage | 2. 250 ppm., twice thrice or four times during flag leaf emergence | 4·5 | ,, |
| | | 3. 250 ppm., twice or thrice during flag emergence | 3. 500 ppm., sprayed once or twice during flag leaf emergence | 5·0 | ,, |

| Crop | Chemical | Concentrations giving complete pollen sterility | | % reduction in seed set | |
|---|---|---|---|---|---|
| | | 1956 | 1957 | 1956 | 1957 |
| Tomato | MH | 1. 100 ppm., sprayed twice | 1. 50 ppm., sprayed twice when treated before first flower opening | 37·9 | 13·7 |
| | | 2. 250 ppm., sprayed twice | 2. 100 ppm., sprayed once or twice at the same stage as in (1) | 36·7 | 18·4 |
| | | | 3. 200 ppm., twice when treated before opening of 2nd flush flowers | .. | 14·2 |
| Onion | Sodium Nucleate | 3% | 3% | 25·4 | Seed set data not available |
| | | 4% | 4% | 9·8 | ,, |

*Cytological effects of the chemicals used.*—Any chemical which is tried in experiments such as the present one, should not possess mutagenic properties. For this purpose the cytological effects of the various chemicals at the concentrations used in this experiment were studied using the standard *Allium* test of Levan (1948). Also, meiosis was studied in the treated plants.

The results showed that, at the concentrations used, MH, thymine, uracil, sodium nucleate and nucleic acid did not induce chromosomal aberrations. Study of meiosis in the treated plants did not reveal any chromosome structural change. Effects on either the nuclear or cytoplasmic constituents can be detected by studying the progeny raised from seeds harvested from treated plants. This is important since many chemicals are known to produce effects of the "dauermodifikation" type (Goldschmidt, 1955). The progenies of wheat plants treated with MH in 1955-56 and grown during 1956-57 were quite normal, vigorous, and fertile just as the untreated parental material.

*Progenies of MH treated plants.*—The $F_1$ generation of the cross between MH treated C. 591 plants and normal N.P. 790 (the cross was made using the treated C.591 as the pistillate parent without emasculation) provided some interesting information. The seeds resulting from 100 and 200 ppm. MH treated plants were nearly all hybrids, as could be easily shown from the tipped nature of the ear of the plants as against the fully bearded nature of C. 591. The hybrid plants were healthy and vigorous and had normal pollen fertility (seed fertility could not be assessed since the plants were damaged by the hailstorm). This clearly indicates that it may be possible to perform crosses in wheat without emasculation. By growing treated plants and the pollen parent selected for crossing in alternate rows it may even be possible to avoid the need

for hand pollination. Work should be undertaken in future to test this possibility. The lax nature of the ear in MH treated plants may facilitate spontaneous cross-pollination. However, critical data concerning the extent of competition of pollen in cases of mixed pollination and of reduction in seed fertility in pollen sterile plants allowed to set seeds under open pollinated conditions, in contrast to those which are hand pollinated with good pollen, are necessary before conclusions concerning the evolution of a suitable technique of pollination could be drawn.

*Method of application.*—Foliar sprays are easily carried out. In fact the application of micronutrients and even major nutrients are now carried out through foliar sprays. Injection method is tedious but was found to be the only effective one in onion. Easier methods will have to be worked out if the results are to be put to practical use.

*Duration for which the effects last.*—In studies of this type it is important to know how long the effects of a treatment would last, particularly in crops like tomato characterised by a continuous period of flowering. During the present study it was found that the effect of MH sprays (optimum concentrations) lasts for about two weeks. This should provide ample time to perform the requisite number of crosses. Since in tomato a single cross would yield more than 100 seeds it would be feasible to undertake studies on a large scale. In wheat, the primary tillers were found to show complete pollen abortion. Hence, they could be used for crossing purposes. In onion also, the injection of chemicals caused pollen sterility in all the flowers of the umbel.

*Reproducibility of effects.*—For a treatment to be precise, the exact concentration, the stage of development of plant when application is to be made and the external conditions of application should be defined. There may be variation in absorption caused by the leaf area and the atmospheric conditions. Also, varietal differences in response to MH have been observed in *Sorghum*. Hence, a suitable dosage may have to be worked out for each variety.

The results of MH application at two different stages of plant growth in tomato are given below:

| Stage of growth | Dosage giving complete pollen sterility | % reduction in seed-setting |
|---|---|---|
| 1. Before first flower opening | (i) 100 ppm. MH, sprayed, twice | 15·9 |
| | (ii) 50 ppm. MH, sprayed twice | 13·7 |
| 2. Before flower opening in second flush | 200 ppm., sprayed twice | 14·2 |

From the above data, it appears that the effective concentration of a chemical will vary with the stage of application; older plants require higher doses as compared to the younger plants. Within the same treatment, however, the results from two replications, were very similar. In set 2, one lot of potted plants and another of field grown plants were treated before the opening of second flush of flowers. The similarity of results from these two treatments indicates that if the stage of growth is precisely defined, a particular concentration will give the same results subject to environmental conditions being similar. By enlarging such a study, the most sensitive stage and the optimum concentration of the chemical can be worked out.

*Hazards of application.*—Large doses of MH have been found to possess deleterious effects on plant growth. No severe hazards to human beings have come to light and any possible danger can be minimized if only low doses are applied. None of the other chemicals appear to possess any adverse effects.

It can, therefore, be concluded that definite possibilities exist for evolving fairly standardized schedules for the induction of pollen sterility in different crop plants. However, even normal physiological processes of plants are subject to environmental variation and the results obtained with such schedules would no doubt show some fluctuation depending upon the prevalent environmental conditions.

## Summary

The effectiveness of maleic hydrazide, tri-iodobenzoic acid, yeast nucleic acid, sodium nucleate, thymine, uracil and F.W.-450 in inducing the selective abortion of pollen was studied in bread wheat, tomato and onion. Maleic hydrazide was found to be the most effective, both in wheat and onion. In wheat, complete pollen sterility occurred in plants treated with MH at 100 ppm. sprayed thrice or 250 ppm. sprayed 1 to 3 times prior to the emergence of flag leaf. In $F_1$ progeny of a cross performed without emasculation between MH treated C. 591 (awned) and normal N.P. 790 (awnless), 80 per cent of the plants proved to be hybrids. The effective concentration of MH was found to rise with an increase in the age of the plant in tomato. Injection of aqueous solutions of sodium nucleate (3 and 4 per cent) led to the complete abortion of pollen in onion. F.W-450, " Gametocide ", depressed the growth of wheat plants and did not cause pollen sterility. From the present results it appears likely that a suitable chemical treatment can be standardised for causing the selective abortion of pollen in wheat, tomato and onion.

## Acknowledgements

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# CHROMOSOME MORPHOLOGY, MICROSPOROGENESIS AND POLLEN FERTILITY IN SOME VARIETIES OF COCONUT

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Critical cytogenetical and breeding investigations on coconut (*Cocos nucifera* L.) have been few. While breeding investigations are handicapped by the long life-cycle of the plant and its highly cross-pollinated nature (Patel, 1938; Menon and Pandalai, 1958), the small size of the chromosomes and technical problems connected with getting dividing cells with well-differentiated cytoplasm and nucleus render cytological studies difficult. Using microtome sections, Santos (1929) studied the development of the microspore and reported the chromosome number n=16 for coconut. This number has been subsequently confirmed by several workers (Janaki Ammal, 1945; Venkatasubban, 1945; Sharma and Sarkar, 1956).

With the evolution of the squash techniques together with the use of several efficient chemical pre-treatments, a detailed study of the morphology of somatic chromosomes has become relatively simple (Sharma and Sarkar, 1955; Nambiar and Upadhya, 1960). Squash techniques also help in getting good preparations of microsporocytes in various stages of meiosis (Nambiar and Swaminathan, 1960). A study of the morphology of somatic chromosomes and meiosis in pollen mother cells was hence undertaken in some varieties of coconut and the results are reported in this paper.

## Materials and Methods

Three coconut varieties—Dwarf Red of local origin, Apricot, a semi-tall strain from the Straits Settlements and an ordinary tall variety of Laccadive islands, belonging to the varietal collection maintained at the Central Coconut Research Station, Kasaragod, were used in the present study. Dwarf Red and Apricot had, on an average, 30 per cent of sterile pollen as judged by stainability in acetocarmine. Laccadive ordinary, on the other hand, had practically all normal pollen (about 5 per cent sterility). For the study of meiosis, flower buds from the inflorescences of these trees were collected 50 to 60 days prior to the opening of the spathe and were fixed in Carnoy's solution (6 absolute alcohol:3 chloroform:1 acetic acid) for 3 hours. The optimum time for fixation was found to lie between 11 a.m. and 1 p.m. The buds were transferred from the Carnoy's mixture to propionic alcohol (1 part of propionic acid staturated with ferric acetate mixed with 3 parts of absolute alcohol). After 24 hours, the buds were transferred to 70 per cent alcohol and stored in a refrigerator. The fixations were made at Kasaragod and the material brought to New Delhi for study. The anthers from the fixed buds were rinsed in 45 per cent acetic acid and then placed for 3 to 5 minutes in a mixture of 5 parts of 45 per cent acetic acid, 4 parts of 45 per cent acetic acid saturated with ferric acetate and 1 part of 1 per cent formalin. This mordanting fluid helped in the intensification of staining. The anthers were then squashed in a drop of aceto-carmine or propino-carmine on a clean glass slide. Analysis was carried out in most cases in temporary preparations but some slides were made permanent for record purposes by passing them through grades of butyl alcohol and then mounting them in neutral balsam.

For the study of somatic chromosomes, the 8-hydroxyquinoline pre-treatment method of Tjio and Levan (1950) was found to be the best. Aesculine pre-treatment

recommended by Sharma and Sarkar (1955) did not give as consistent or as good results as the schedule of Tjio and Levan (1950). Treatment for 2-1/2 to 3 hours with 0·002 M 8-hydroxyquinoline followed by fixation and hydrolysis in NHCl and squashing in acetic-orcein gave preparations with well-spread chromosomes of considerable clarity (Nambiar and Upadhya, 1960). The root tips used in the study of somatic chromosomes were collected from the seedlings obtained from Kasaragod and grown at the I.A.R.I., New Delhi. The cells were in active mitosis between 12 noon and 1 p.m. during the months of October and November under Delhi conditions.

## Experimental Results

I. *Somatic Chromosomes.*—Chromosome morphology was studied in an ordinary tall variety of Coconut. All cells with well-spread chromosomes were carefully drawn with a Camera-lucida and each chromosome from the drawings of 5 clear cells was measured carefully with the help of dividers. Measurements of chromosomes from each metaphase plate were then converted into relative lengths, i.e. the length of each chromosome expressed as a percentage of the total chromatin length of the complement. The chromosome index (short arm/long arm ratio) was also determined for every chromosome.

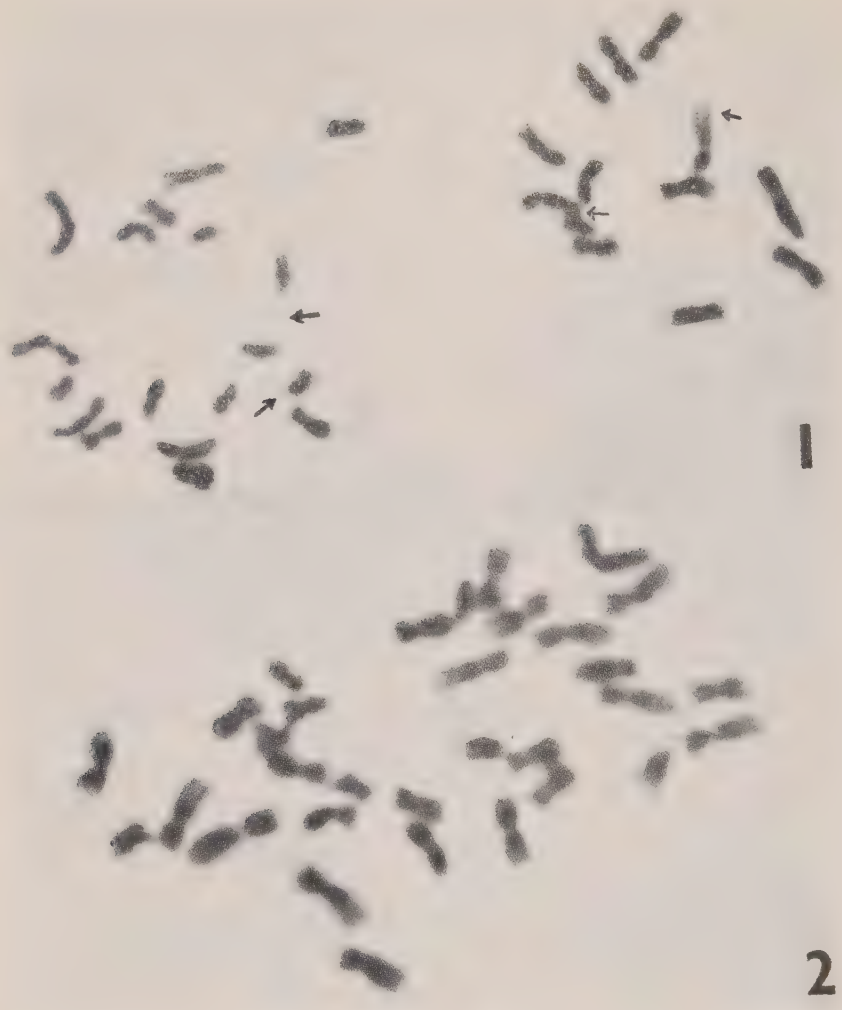
The values obtained from the 5 metaphase plates did not differ from each other appreciably and hence the mean value for each chromosome was used for preparing the idiogram (Text Fig. 1). In the satellite-bearing chromosomes, the length of the satellite/length of the arm bearing the satellite was used to determine the size of the satellite.

In the idiogram, the gap of the primary and secondary constrictions has been kept constant. By using the chromosome index and relative lengths, errors which may arise from possible differential contraction among the same chromosomes in different cells have been avoided. The data relating to the individual chromosomes are given in Table 1. Photomicrographs of two somatic metaphase cells are given in Figures 1 and 2.

Table 1

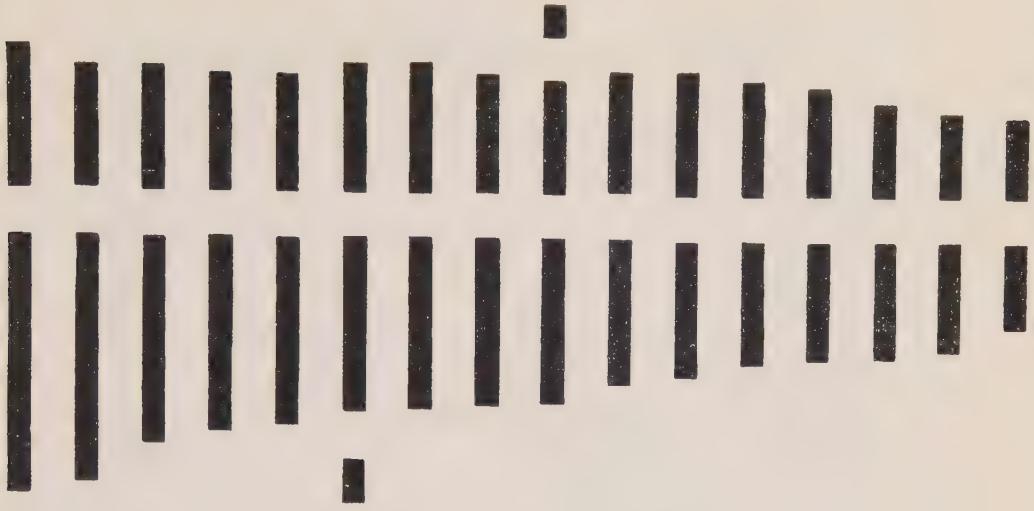
*Relative length and index of the chromosomes of a Tall variety of Coconut*

| Chromosome No. | Relative length (in microns) | | | S.A./L.A. Index | Satellite | |
|---|---|---|---|---|---|---|
| | Long arm | Short arm | Total | | R.L. | Index |
| 1. | 5·88 | 3·23 | 9·11 | 0·55 | | |
| 2. | 5·57 | 2·79 | 8·36 | 0·50 | | |
| 3. | 4·65 | 2·81 | 7·46 | 0·59 | | |
| 4. | 4·39 | 2·65 | 7·04 | 0·59 | | |
| 5. | 4·25 | 2·66 | 6·91 | 0·61 | | |
| 6. | 4·02 | 2·89 | 6·91 | 0·71 | 1·01 | 0·36 |
| 7. | 3·87 | 2·93 | 6·80 | 0·74 | | |
| 8. | 3·82 | 2·67 | 6·49 | 0·68 | | |
| 9. | 3·76 | 2·58 | 6·34 | 0·66 | 0·76 | 0·27 |
| 10. | 3·29 | 2·72 | 6·01 | 0·81 | | |
| 11. | 3·12 | 2·79 | 5·91 | 0·88 | | |
| 12. | 2·83 | 2·57 | 5·40 | 0·89 | | |
| 13. | 2·73 | 2·45 | 5·18 | 0·88 | | |
| 14. | 2·67 | 2·10 | 4·77 | 0·77 | | |
| 15. | 2·49 | 1·83 | 4·32 | 0·72 | | |
| 16. | 1·97 | 1·79 | 3·76 | 0·89 | | |




Figs. 1 & 2. Somatic chromosomes of a tall variety of coconut. The sat-chromosomes are marked with arrows.

A study of the idiogram (Text Fig. 1) an the data in Table 1 would show that (1) two pairs of chromosomes are much longer in comparison with the others; (2) two pairs bear satellites; (3) three pairs are relatively short and (4) all chromosomes have either sub-median or subterminal centromeres. The longer chromosomes were in general more heterobrachial than the shorter ones. The chromosomes bearing satellites occupied the 6th and 9th positions in order of the total chromosome length. The satellite was present on the long arm in chromosome VI, and on the short arm in chromosome IX. Sharma and Sarkar (1956) have also reported the presence of two pairs of sat-chromosomes in the coconut variety studied by them. They found that




Text Fig. 1. Idiogram of a tall variety of coconut.

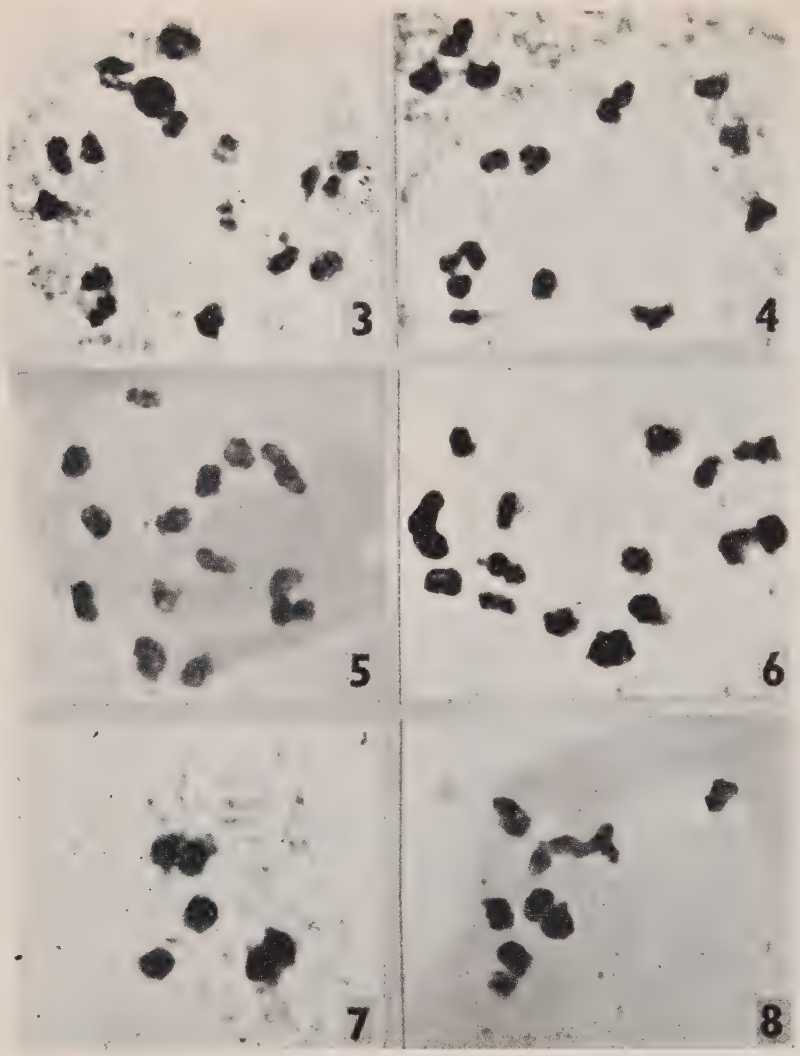
in these two pairs, the primary and secondary constrictions were nearly median and sub-median in position. This, however, was not found to be the case in the variety studied by us.

II. *Meiosis:* (a) *Laccadive ordinary.*—Meiosis was regular in this variety (Table2). There were 16 bivalents at diakinesis and MI (Figs. 3 to 5). Two bivalents were attached to the nucleolus at diakinesis. There were considerable size differences among the bivalents; one bivalent was particularly large and three others were small (Fig. 3). On an average, 77 per cent of the bivalents of a cell had chiasmata in both the arms and 23 per cent were of the rod type with a single chiasma in one of the arms. Among the ring bivalents, 8 per cent had 3 chiasmata each. Anaphase I and subsequent stages were normal. At the sporad stage, a cell with 5 spores was the only abnormality observed (Table 5).

Table 2

*Chromosome associations at Diakinesis and MI*

| Variety and Tree No. | No. of P.M.C. studied | Mean frequency per cell | | Mean No. of Xta | |
|---|---|---|---|---|---|
| | | Quadrivalent | Bivalent | per cell | per bivalent |
| Apricot (X 1/62) | 49 | 0·041 | 15·918 | 26·5 | 1·74 |
| Dwarf Red (XI/75) | 54 | 0·019 | 15·962 | 29·03 | 1·84 |
| Laccadive Ordinary (XI/27) | 51 | .. | 16·00 | 30·57 | 1·91 |




FIGS. 3 to 5. Meiosis in Laccadive Ordinary.

FIG. 3. Diakinesis with 2 bivalents attached to the nucleolus. Note the difference in the size of the bivalents.
FIGS. 4 & 5. Metaphase I with 16 bivalents.

FIGS. 6 to 8 Meiosis in Apricot.

FIG. 6. Metaphase I. $1_{IV}+14_{II}$
FIG. 7. A cell with 6 bivalents.
FIG. 8. A cell with 10 bivalents.

(b) *Apricot.*—Besides microsporocytes with regular meiosis, several showing various types of abnormalities were also observed in this variety. In two cells at diakinesis and MI, one quadrivalent and 14 bivalents were observed (Fig. 6). The mean number of chiasmata per bivalent in this variety was lower than that recorded in Laccadive Ordinary (Table 2). The nucleolus, which normally disappears at the end of prophase and reforms at telophase, was found to persist in some cells both during MI and later stages (Figs. 9 and 10). Another interesting abnormality was the occurrence of microsporocytes with varying chromosome numbers in the same anther (Figs. 7 and 8). Such " chromosome mosaic " cells constituted 12·3 per cent of the cells studied at MI (Table 3) and should owe their origin to disjunctional abnormalities during pre-meiotic mitosis.

TABLE 3

*Frequency of P.M.Cs with different Chromosome Numbers*

| Variety | No. of cells with n= | | | | | Percentage of Normal cells |
|---|---|---|---|---|---|---|
| | 6 | 8 | 10 | 14 | 16 | |
| Apricot .. | 2 | 1 | 1 | 2 | 43 | 87.7 |
| Dwarf Red .. | | | | | 54 | 100 |
| Laccadive Ordinary .. | | | | | 51 | 100 |

At anaphase I, a dicentric bridge and an acentric fragment were observed in two cells, indicating heterozygosity for an inversion (Fig. 11). Lagging chromosomes were seen at anaphase I and micronuclei occurred at telophase (Table 4; Fig. 12). At the sporad stage, besides normal tetrads, sporads with 1, 5, 6, 7 and 8 spores respectively were also seen (Table 5; Figs. 13 and 14).

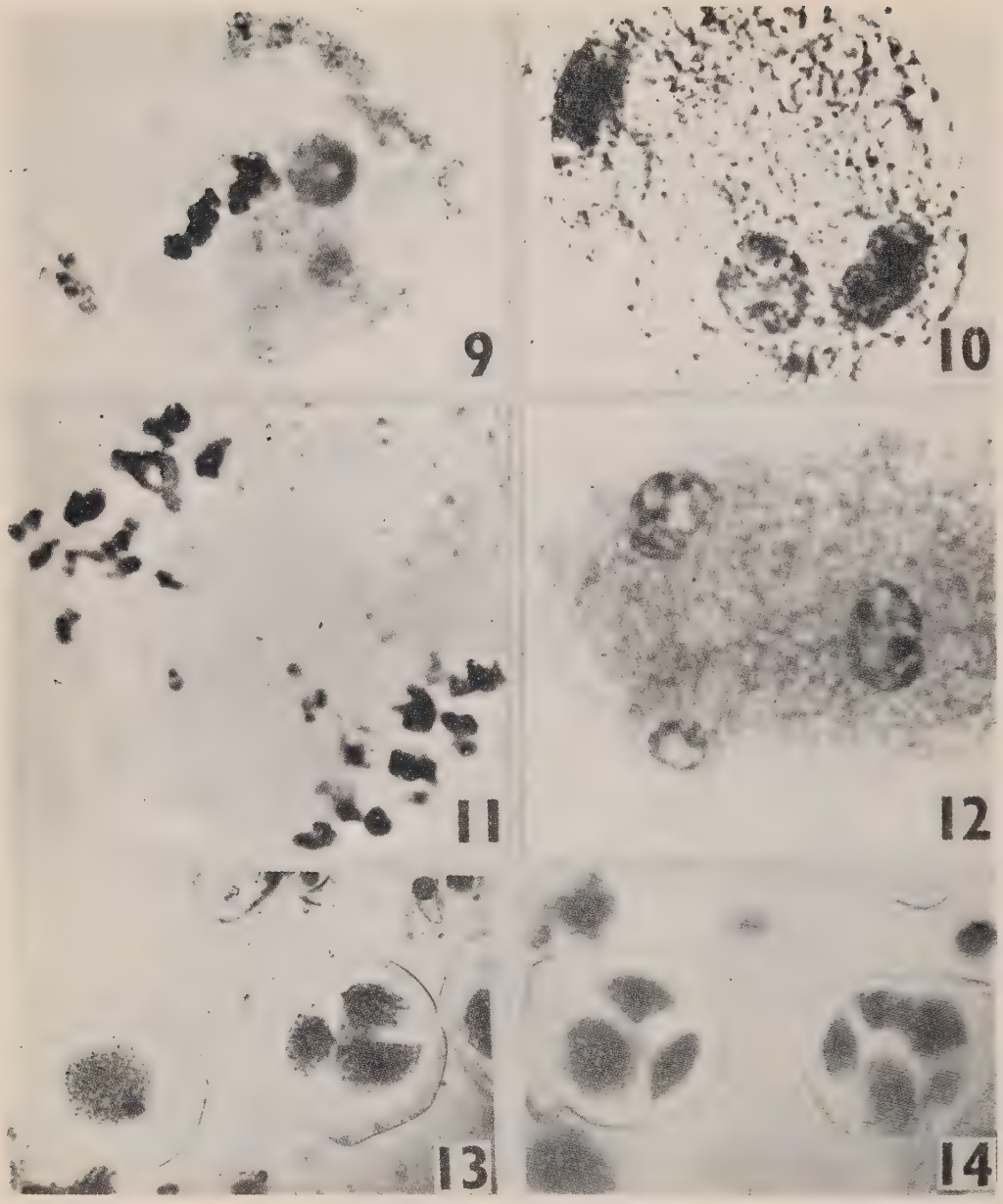
(c) *Dwarf Red.*—In this variety also, several cells with meiotic irregularities were observed. These included the occurrence of (a) one quadrivalent in a cell at MI; (b) inversion bridge at AI, (c) laggards at AI and micronuclei at Telophase I and II and (d) sporads each with 5, 6, 7 and 8 spores respectively (Tables 2, 4 and 5).

Persistent nucleoli and chromosome mosaic cells which were noted in Apricot were, however, not observed in this variety.

TABLE 4

*Irregularities at AI and later stages*

| Variety | Stage | No. of P.M.Cs. studied | Normal P.M.Cs. | No. of cells with | | |
|---|---|---|---|---|---|---|
| | | | | Inversion bridge | Laggard | Micro-nuclei |
| Apricot .. | A-I | 46 | 29 | 2 | 15 | |
| | A-II | 20 | 20 | .. | .. | .. |
| | T-I | 30 | 21 | .. | .. | 9 |
| | T-II | 25 | 23 | .. | .. | 2 |
| Dwarf Red .. | A-I | 24 | 18 | 1 | 5 | .. |
| | A-II | 25 | 25 | | | |
| | T-I | 25 | 23 | .. | .. | 2 |
| | T-II | 25 | 24 | .. | .. | 1 |




Figs. 9 to 14. Meiosis in Apricot.

Fig. 9. Metaphase I with a persistent nucleolus.
Fig. 10. Telophase I with a nucleolus persisting in the plasm.
Fig. 11. Anaphase I with a dicentric bridge and an acentric fragment.
Fig. 12. A micronucleus.
Fig. 13. A monad and a tetrad.
Fig. 14. A triad and a pentad.

TABLE 5

*Abnormalities at the sporad stage*

| Variety | No. of sporads studied | Monad | Tetrad | Pentad | Hexad | Heptad | Octad | % of normal sporads |
|---|---|---|---|---|---|---|---|---|
| Apricot .. | 103 | 4 | 74 | 14 | 6 | 2 | 3 | 71·8 |
| Dwarf Red Laccadive | 214 | 0 | 197 | 8 | 4 | 4 | 1 | 92·1 |
| Ordinary.. | 122 | 0 | 121 | 1 | 0 | 0 | 0 | 99·2 |

III. *Pollen fertility.*—The frequency of pollen which stain well with acetocarmine was calculated for trees of all the three varieties. Pollen fertility measured in this way was found to vary among the different inflorescences of a spadix and also with the season. However, from data collected over two years, it was clear that certain varieties always tended to possess a higher frequency of sterile pollen than the others. Thus, while Apricot and Dwarf Red had on an average only 70 per cent of well-stained pollen, Laccadive ordinary had over 95 per cent of such pollen. Apricot and Dwarf Red are, therefore, partially pollen sterile.

## DISCUSSION

The karyotype of the coconut variety studied by us was characterised by a greater proportion of chromosomes with sub-median centromeres and a considerable difference in size between the largest and the smallest chromosomes of the set. From the meiotic observations as well as from the data of Sharma and Sarkar (1956), it seems likely that most varieties of *Cocos nucifera* may show these features in their karyotypes. The use of karyotype symmetry-asymmetry in the study of species evolution is well-known and in the Ranunculaceae, tribe Helleboreae, Levitsky (1931) showed that the most primitive species tend to have chromosomes possessing median centromeres and of nearly equal size. In order to study the possible connection between increasing asymmetry and other characteristics of the plants concerned, Stebbins (1958) has proposed a new classification of types of symmetry. Similar studies in the Tribe *Cocoineae*, to which *Cocos nucifera* belongs, may yield interesting information.

Varying degrees of pollen sterility have been reported by several workers in varieties of coconut. Patel (1938) found about 25 per cent of sterile pollen grains in six trees while Aldaba (1921) observed 3 to 33 per cent sterility in some varieties in the Philippines. Sharma and Sarkar (1956) have stated, without specifying the percentage of sterility and the name of the variety studied by them, that a high percentage of pollen sterility occurs in coconut. They have further suggested that the " high percentage of pollen sterility stands against the assumption that sexual reproduction becomes effective in the production of fruits, which are abundant in this

species". Their suggestion that pollination in coconut may provide a stimulus for apomictic reproduction, however, finds no support in the results of the breeding experiments conducted at the coconut research centres in several countries. Aldaba (1921) has estimated that each male flower contains about 272 million pollen grains. Also, the extent of pollen sterility varies among the different flowers of an inflorescence and with the environmental conditions. Hence, for getting an estimate of pollen sterility in a tree it is necessary to carry out detailed quantitative studies over a period of time. In a study lasting for over two years, it was found that the varieties Apricot and Dwarf Red had on an average about 30 per cent pollen sterility while the variety Laccadive Ordinary had only 5 per cent sterility. It is thus clear that coconut trees belonging to different varieties show different degrees of pollen sterility.

A study of microsporogenesis showed that several abnormalities occurred in the varieties Apricot and Dwarf Red. Thus, there were cells with (a) one quadrivalent at diakinesis and metaphase I suggesting heterozygosity for a reciprocal interchange; (b) a dicentric bridge and an acentric fragment at anaphase I indicating heterozygosity for an inversion; (c) nucleoli which persist at metaphase and anaphase; (d) pollen mother cells with varying chromosome numbers; (e) lagging chromosomes at the first and second anaphase; (f) micronuclei at telophase and (g) sporads with varying number of spores. There was also a reduction in chiasma frequency in Apricot and Dwarf Red in comparison with Laccadive Ordinary. Meiosis was regular in Laccadive Ordinary, the only abnormality observed being a pentad at the sporad stage. The data thus suggest that aberrations during meiosis may be responsible for the pollen sterility observed in the varieties Apricot and Dwarf Red. From the occurrence of "chromosome mosaic" cells, it is obvious that abnormalities in cell division also occur during premeiotic stages. The frequency of cells with abnormalities as well as the types of aberrations found were more in Apricot, which is a semi-tall variety, than in Dwarf Red, a pure dwarf strain.

It is of interest that both Apricot and Dwarf Red have dwarf characteristics while Laccadive Ordinary is a tall variety. The dwarf coconut is small in stature and commences flowering as early as the third year after planting and comes to regular bearing in the ninth year. The origin of the dwarf varieties of coconut is not known with any degree of certainty, although several authors have suggested that the dwarfs might have arisen as mutants from the common tall plants (Menon and Pandalai, 1958). While the tall varieties are largely obligatorily cross-pollinated, the dwarfs can undergo self-pollination owing to the overlapping of the female and male phases in the same inflorescence. The various distinctive features of typical dwarf and tall varieties based on data from previous and the present studies are summarised in Table 6.

Because of the several undesirable features they possess, most coconut research workers have advised against the cultivation of dwarf palms on a plantation scale. Some dwarf lines seem to be more vigorous than others and some have good quality of copra but the general experience with dwarf varieties is not encouraging (Tammes, 1955). Crosses between the ordinary coconut and the dwarf varieties occur in nature and have also been made artificially. As a result, all gradations between the two contrasting categories outlined in Table 6 occur. In general, the hybrids have an early bearing habit, nuts of intermediate size and a cross-pollinating nature. Harland (1957) has suggested that the dwarf varieties, by virtue of their being largely self-pollinated and reasonably homogeneous, can be used for identifying the most prepotent males. Haldane (1958) has pointed out that if dwarfness is mainly due to a single gene, the introduction of this gene into the various races of coconut may be desirable since, in some important characters, the hybrids between tall and dwarf strains are superior to the tall parents.

TABLE 6

*Contrasting characteristics of Tall and Dwarf coconut*

| Character | Tall palm | Dwarf palm |
|---|---|---|
| Height .. | .. 15 to 18 meters or more | 5 to 10 meters |
| Years needed for first bearing .. | .. 8 to 10 | 3 to 5 |
| Crowns .. | .. Large | Small |
| Plant parts .. | .. Large | Small |
| Vigour .. | .. Healthy | Weak |
| Pollination .. | .. Largely cross-pollinated | Largely self-pollinated |
| Average life .. | .. 80 to 90 years | 35 to 40 years |
| Nuts .. | .. Large | Small |
| Appearance of copra | .. Good | Poor |
| Percentage of rubbery pieces in copra | .. 0–10 | 10 to 92 |
| Nuts per picul of copra | .. 200-284 | 350 to 513 |
| Pollen fertility | .. High | Varying degrees of sterility |
| Meiotic behaviour | .. Regular | Irregular |

From our limited cytological studies, it would be premature to offer suggestions concerning the possible mode of origin of the dwarf palms. It is, however, difficult to refrain from comparing the cytological characteristics of the two semi-dwarf and dwarf varieties studied by us with the results reported in inbred rye by Lamm (1936), Prakken and Müntzing (1942), Müntzing and Akdik (1948) and Rees (1956). All these authors have clearly shown that the behaviour of the chromosomes is less efficient in inbred rye plants than in population plants. The unbalance caused by homozygosity in normally outbred populations may show up at the chromosomal level in different ways in different homozygotes. The occurrence of " chromosome mosaic " cells at meiosis and a poor development of the endosperm in dwarf coconuts suggests that the efficiency of the chromosome mechanism is affected in somatic, gametic and endosperm cells.

If further studies confirm our findings, it would appear that the dwarf and semi-dwarf coconuts occurring in nature are the products of various generations of inbreeding of the normal strains. The following two points appear to support this possibility. First, the tall and dwarf varieties differ in not one but a series of characters (Table 6), all of which seem to be the consequence of different degrees of reduced cell growth and aberrant cell division. Secondly, the $F_2$ progeny of a dwarf $\times$ tall cross gave a wide range of segregates and it was not possible to interpret the data on any simple genetic basis (Tammes, 1955). Distinct varieties of dwarf coconut occur in Malaya, Philippines, Fiji islands, Ceylon, Viet Nam and India. Also, trees with characters ranging between the two extremes described in Table 6 occur in these countries. It is possible that these dwarfs and semi-talls have arisen through inbreeding among different tall parents. In dwarfs which have attained equilibrium conditions with regard to growth characters inbreeding depression may not occur on selfing. Also, the wide extent of variability found among the dwarfs with regard to yield and quality of nuts would suggest that different varieties of coconut may respond to selfing in different ways.

It is of considerable interest that meiosis was more regular in the pure dwarf variety studied by us than in the semi-tall one. This suggests that meiotic aberrations may be relatively more frequent in early generation inbreds. It is possible that some of the stable and evolved dwarfs might have reached equilibrium conditions with regard to cytological behaviour also. While the results of the present study suggest that the dwarfs now occurring in nature might have evolved over a period of several thousand years through inbreeding among the normal coconut plants, an initial mutation, if any, responsible for their origin might be concerned with the bringing about of an overlapping of the male and female phases of the inflorescences thereby rendering self-pollination both possible and predominant. However, self-pollination can occur in the tall varieties during certain seasons (Patel, 1938; Dwyer, 1938) and a mutation facilitating this process is not a pre-requisite for inbreeding to occur. Alternatively, a situation analogous to that described by Harland (1955) in the maize strains of Trinidad may be responsible for the evolution of stable self-pollinating dwarfs.

Apart from the theoretical interest aroused by the question whether the dwarf palm owes its origin to inbreeding facilitated by the imposition of a self-pollinating device, or due to an one-step mutation, an understanding of the problem is extremely important from the breeding point of view. If the early bearing nature of the dwarf palms is only the consequence of a depressed vegetative vigour, the transference of this character through tall × dwarf crosses can probably be achieved only at the expense of the longevity and prolonged high productivity of the plant. This by itself may not be a serious handicap since by re-planting at more frequent intervals, a high yielding population can be maintained. On the other hand, if the dwarf palms represent homozygous genotypes evolved from tall strains as a result of inbreeding, there may be excellent scope for studying the manifestation of heterosis in crosses among distinct dwarf strains. Unfortunately, most of the breeding work carried out so far relates to only reciprocal crosses between tall and dwarf varieties or among tall strains. The only instance reported in literature of a cross between two distinct dwarfs is the one made by Marechal (1928) between a Malayan dwarf and N'uleka, a dwarf strain from the Fiji islands. The $F_1$ hybrids of this cross have shown outstanding performance and have been much sought after in Fiji (Parham, 1953). In the light of our studies, it seems desirable that this line of work should be taken up at coconut breeding centres without further loss of time. The technique suggested by Harland (1957) to identify prepotent males can be used to estimate the combining ability of different parents in dwarf × dwarf crosses.

## Summary

1. The karyotype of a tall variety of coconut was studied using relative length, and arm ratio and the position and number of secondary constrictions as criteria for classification. A majority of chromosomes had sub-median centromere and there were considerable differences in length. Two pairs were satellited.

2. Microsporogenesis was studied in trees of Laccadive Ordinary, Apricot and Dwarf Red, which are tall, semi-tall and dwarf varieties of coconut respectively. Meiosis was regular in Laccadive Ordinary while irregularities such as heterozygosity for translocations and inversions, reduced chiasma frequency, persistent nucleoli, chromosome mosaic cells, lagging chromosomes at AI and AII, micronuclei at telophase and sporads with varying number of spores were observed in Apricot and Dwarf Red. The frequency of aberrant cells was higher in Apricot.

3. Laccadive Ordinary, Apricot and Dwarf Red had on an average 5, 30 and 30 per cent of sterile pollen respectively in studies carried out over a period of two years.

4. The cytological behaviour of the semi-tall and dwarf varieties resembles that of inbred rye. It is suggested that the dwarf coconut may owe its origin to inbreeding facilitated by the imposition of a self-pollinating device. The dwarf palms may, therefore, offer excellent material for evolving high yielding hybrid trees. The only instance of a cross between two distinct dwarfs reported in the literature supports this view.

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# STUDIES ON THE INDUCED POLYPLOIDS OF HORSE GRAM

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To evolve better varieties, tetraploidy has been induced in several seed and forage legumes. While in none of the seed legumes like gram, pea, arhar, green gram etc., the yield could be increased so far, tetraploids of a few forage legumes have been put to practical use. Notable among these are red clover (Levan 1942, Müntzing, 1951), and alsike clover (Armstrong and Robertson, 1956). Hutton and Peak (1954) in one variety of subterranean clover, and Sikka *et al.* (1958) in Egyptian clover also reported higher forage yield in the tetraploids.

Horse gram, also known as *kulthi* bean, is an important seed-cum-forage legume of South India. The crop can be grown on practically all types of soil and is hardy and drought resistant. The plants are self-fertilized, the chromosomes are small and few. The chromosome number has been reported by Rau (1929) as 2n=24, but by Sen and Vidyabhushan (1959) as n = 10 and 2n = 20.

As there has been no report on the effects of tetraploidy in this crop, this project was undertaken. Several varieties were included since differential response among the varieties due to tetraploidy in morphological and physiological characters have been observed by most of the workers. The importance of broad-basing the tetraploid breeding programme by including several diploid varieties has also been emphasised (Ramanujam and Parthasarathy, 1953) and it has been shown that by selection from a wider range of induced tetraploid varieties and by subsequent recombination-breeding considerable improvement can be made in autogamous plants like flax (Kuhk, 1943), barley (Müntzing, 1948) etc.

## MATERIALS AND METHODS

The following seven varieties, collected from different Agricultural Stations in India, were used in the present study: Dharwar, Bijapur, Sholapur, Hyderabad, Coimbatore 35, Ashy Hebbal and Coimbatore 4. The first three varieties are early in flowering and the latter four are late. The variety Dharwar has conspicuously larger plant parts and fewer branches.

To induce tetraploidy, pre-soaked seeds and apical buds of about one-week-old seedlings growing in the field were treated with aqueous solution of colchicine. The concentrations and durations for the seed treatments ranged from 0·01 to 0·25 per cent and ½ to 24 hours, and for the apical-bud treatment from 0·1, 0·25 and 0·5 per cent for 3 and 9 hours.

In the initial stages, the affected plants, which were slow growing with broader, thicker, darker green and variously deformed leaves, were marked. When these plants started flowering suspected polyploid sectors were spotted on the basis of flower size and pollen sterility and in some cases confirmed from chromosome counts. The effects of tetraploidy on various morphological, anatomical, physiological and cytological aspects were studied from the $C_2$ generation plants.

For the study of mitotic chromosomes, root tips were pretreated with saturated solution of paradichlorobenzene for about 1½ hours at 12-15° C and smeared in 1 per cent aceto-orcein after warming in a mixture of 2 per cent aceto-orcein and 1N HCl (9:1) Fixation of the material in 1:2 proponic-alcohol with a pinch of ferric acetate for about 2 hours after pretreatment improved stainability of the chromosomes

considerably. For meiotic preparations, flower buds were fixed between 11 a.m. and 2 p.m. in acetic-alcohol (1:3) for 24 hours, and the anthers were smeared following the iron-aceto-carmine technique. Both mitotic and meiotic studies were made from temporary preparations. The slides were later made permanent by removing the cover slip in normal-butyl alcohol and mounting in neutral canada balsam.

## Experimental Results

*Colchicine treatment.*—The roots of the seedlings from treated seeds grew slowly and developed slight swellings. Although in a majority of the seedlings the root gradually resumed growth, the stem apex failed to grow and consequently the survival was very low. Inspite of the wide range of treatments, not a single plant with polyploid sectors could be detected among the surviving plants.

On the other hand, in the apical-bud treatments not only the survival, but also the number of colchiploid plants were appreciably high. Except in a few seedlings in the long duration treatments, i.e. 0·25 and 0·5 per cent - 9 hours, the main axis of the treated seedlings usually resumed growth. Although polyploid plants were obtained in almost all the six treatments in the seven varieties, 0·25 per cent-9 hour, and 0·5 per cent-3 hour treatments gave maximum percentage of colchiploids. Most of the affected plants were chimaeras of diploid and tetraploid sectors from which pure tetraploids were obtained in the next generation.

*Germination.*—Germination of both diploid and tetraploid seeds in the field commenced between third and fourth day, but continued upto eighth-day, owing to the presence of variable proportion of seeds in diploid as well as in tetraploid, which take longer time to imbibe water. The germination in diploids ranged from 90-95 per cent, but was lower by about 5-15 per cent in tetraploids.

*Stem and leaves.*—Mean height, number of branches, dry weight and thickness of stem of ten full-grown plants selected at random are given in Table 1.

The mean height of diploid and tetraploid in all varieties remained more or less unchanged till 30th day. But between 30-60 days the increase in height of diploids was appreciably more except in Sholapur and Hyderabad. From 60 days onwards the growth in the diploids gradually slowed down, and by 90 days the main axes of the plants had practically ceased to elongate. The tetraploids, on the contrary, grew faster than diploids during this period and eventually surpassed the height of diploids except in two varieties.

The mean number of branches per plant was less in tetraploids of all varieties from the early stages, although at the end Sholapur and Hyderabad tetraploids had equal number of branches as the corresponding diploids.

Dry weight of tops was determined in an early, and a late variety. The tetraploids of Dharwar had slightly more dry weight all along though the differences were not significant at any stage. On the contrary, in Coimbatore 4 the dry weight of tetraploids was less at the different stages but more at the end, and here also the differences were not significant.

Basal diameter of the stem of full-grown plants was greater in tetraploids of all varieties. The varieties, however, exhibit considerable differences in the increase, which was more in Dharwar, Hyderabad, and Sholapur; moderate in Ashy Hebbal and Coimbatore 4; and negligible in Bijapur and Coimbatore 35.

Cross sections of the mature stem of diploid and tetraploid plants at corresponding regions having nearly equal thickness reveal the following points. The cortical and phloem regions were more or less of similar size. Secondary xylem (wood) was less compact in tetraploids due to wider medullary rays. The width of the secondary xylem cylinder did not exhibit any deviation in tetraploids from diploids with equal basal diameter in the first internodal region. But in the second internodal region

TABLE 1

*Mean height, number of branches, dry weight, thickness of stem of full-grown plants and leaf size*

| | | Dharwar | Bijapur | Sholapur | Hyderabad | Coimb. 35 | Ashy Hebbal | Coimb. 4 |
|---|---|---|---|---|---|---|---|---|
| 1. Height of the plant on 105th day (cm.) | 2n | 46·9 ±7·27 | 58·2 ±6·63 | 62·2 ±6·20 | 49·8 ±5·45 | 70·5 ±8·00 | 75·4 ±6·99 | 79·6 ±6·90 |
| | 4n | 40·8 ±5·70 | 82·7 ±10·60 | 62·5 ±6·80 | 74·5 ±8·83 | 75·1 ±8·90 | 94·7 ±10·62 | 106·0 ±12·15 |
| 2. No. of branches on 90th day | 2n | 5·9 | 9·2 | 7·8 | 10·2 | 10·8 | 12·8 | 14·1 |
| | 4n | 4·5 | 7·9 | 8·0 | 10·4 | 8·8 | 11·5 | 13·8 |
| 3. Dry weight of tops on 105th day (gm.) | 2n | 12·03 ±1·80 | | | | | | 56·4 ±18·4 |
| | 4n | 17·60 ±3·66 | | | | | | 66·6 ±14·4 |
| 4. Thickness of stem (mm.) | 2n | 3·69 ±0·28 | 4·01 ±0·31 | 3·78 ±0·30 | 4·14 ±10·25 | 3·60 ±0·33 | 4·10 ±0·17 | 5·30 ±0·28 |
| | 4n | 4·66 ±0·31 | 4·29 ±0·27 | 5·00 ±0·31 | 5·63 ±0·39 | 3·90 ±0·26 | 4·70 ±0·19 | 5·95 ±0·30 |
| *Fifth leaf:* | | | | | | | | |
| 1. Length of the rachis (cm.) | 2n | 4·18 ±0·27 | 4·31 ±0·18 | 3·81 ±0·21 | 4·94 ±0·35 | 6·17 ±0·35 | 5·09 ±0·27 | 6·86 ±0·28 |
| | 4n | 4·22 ±0·38 | 4·76 ±0·22 | 4·55 ±0·21 | 5·12 ±0·18 | 5·66 ±0·30 | 5·01 ±0·21 | 6·77 ±0·23 |
| 2. Length of the terminal leaflet (cm.) | 2n | 7·03 ±0·18 | 5·42 ±0·15 | 5·25 ±0·23 | 5·41 ±0·12 | 5·99 ±0·24 | 5·86 ±0·16 | 6·85 ±0·17 |
| | 4n | 6·99 ±0·25 | 5·51 ±0·15 | 5·38 ±0·20 | 5·54 ±0·13 | 5·38 ±0·15 | 5·58 ±0·27 | 6·35 ±0·24 |
| 3. Width of the terminal leaflet (cm.) | 2n | 3·84 0·15 | 2·78 0·10 | 3·22 0·14 | 3·22 0·14 | 4·19 0·21 | 3·61 0·17 | 3·78 0·09 |
| | 4n | 4·38 0·30 | 3·71 0·15 | 4·22 0·20 | 4·22 0·14 | 4·50 0·19 | 4·12 0·20 | 4·58 0·15 |

the secondary xylem was considerably narrowed down in tetraploid stem. The walls of xylem fibres were less thickened and the lumen of the xylem vessels was increased in tetraploids resulting in the increase of cell size. The medullary region was wider in the tetraploids.

In the tetraploids, the leaf rachis were thicker and more or less of the same length. The width of the leaflets increased significantly, but the length remained unaffected. The leaves were darker green in colour and appeared thicker.

The stomatal guard cells were increased both in length and width in tetraploids of all varieties. Percentage increase was usually more than in width. The frequency

of stomata per unit area was significantly reduced in tetraploids, which have 50-75 per cent of diploid frequency.

A cross section through the region between veinlets of the leaf shows that the cells of the upper epidermis were larger and broader with pronounced convexity on either side of the periclinal wall. The palisade was rather compact comprising of two layers. Spongy parenchyma was narrower than palisade and ranged from one to two layers in thickness.

The thickness of the leaf in tetraploid was increased by 33·3 per cent in Dharwar, and 40·9 per cent in Hyderabad which was due to larger size of the component cells. All the three regions—epidermis, palisade, and spongy tissues—contributed to the increase in thickness of tetraploid leaves. The increase in cell size was seen not only in length but also in width as the measurements on the upper epidermal cells suggest (Table 2).

TABLE 2

*Comparative measurements on the different tissues of diploid and tetraploid leaves*

| Variety | | Total thickness of leaf blade in μ | Thickness of different layers in μ | | | |
|---|---|---|---|---|---|---|
| | | | Upper epidermis | | Palisade layers | Spongy tissue |
| | | | Length | Width | | |
| Dharwar | 2n | 152·0–182·4 | 30·4–41·8 | 30·4– 76·0 | 57·0– 87·4 | 38·0–58·2 |
| | 4n | 201·4–231·8 | 45·6–76·0 | 45·6– 95·0 | 68·4– 76·0 | 45·6–57·0 |
| Hyderabad | 2n | 152·0–178·6 | 26·6–38·0 | 38·0– 95·0 | 68·4– 98·8 | 38·0–49·4 |
| | 4n | 239·4–266·0 | 41·8–64·6 | 57·0–114·0 | 95·0– 133·0 | 57·0–76·0 |

*Flower*.—Flowering was delayed in the tetraploids of all varieties which was comparatively more in the early varieties. In the tetraploid flowers all the floral parts were larger, though the intensification of different structures varied markedly. In general, the increase in width was more than increase in length, and in the narrower parts like wing and keel the percentage increase in width was considerably more.

The pollen from tetraploid plants present a striking contrast to that of diploid ones. While major portion of the pollen from tetraploid plants comprised of grains with four germ pores those of diploids comprised of ones with three germ pores. The pollen of tetraploid plants have greater range in size and the mean was increased in all varieties, although it was not significant in Ashy Hebbal, Sholapur and Coimbatore 4. Pollen stainability in all the tetraploid varieties was rather good.

*Pod and seed*.—The number of seeds per pod was variable both in diploid and tetraploid. Pods with one or two seeds were in preponderance in tetraploids, 3-seeded pods were in nearly equal proportion in diploid and tetraploid, 4 and 5-seeded pods were in preponderance in diploids, and 6 and 7-seeded pods were almost absent in tetraploids. The mean number of pods per plant was considerably variable among the varieties. At diploid level the numbers ranged from 74 in Dharwar to 253 in Coimbatore 4. Tetraploids of Hyderabad, Ashy Hebbal, Sholapur and Coimbatore

TABLE 3

*Mean flowering time, size of the standard petal, pollen diameter and pollen fertility*

| | | Dharwar | Bijapur | Sholapur | Hyderabad | Coimb. 35 | Ashy Hebbal | Co.4 |
|---|---|---|---|---|---|---|---|---|
| 1. Flowering time in days after sowing | 2n | 40·2 ±0·97 | 40·8 10·66 | 39·9 ±0·72 | 52·4 ±0·82 | 51·4 ±1·09 | 50·5 ±0·81 | 52·4 ±0·77 |
| | 4n | 50·5 ±1·36 | 46·1 ±1·63 | 47·5 ±1·16 | 55·6 ±1·05 | 55·0 ±1·11 | 54·1 ±1·16 | 55·9 ±1·55 |
| 2. Standard Length × Width (mm.) | 2n | 12·0 × 9·75 | 11·15 × 9·15 | 11·35 × 0·40 | 11·2 × 9·3 | 11·15 × 9·3 | 11·7 × 9·85 | 11·65 × 9·70 |
| | 4n | 12·9 × 11·35 | 11·65 × 10·20 | 11·65 × 10·45 | 11·2 × 10·3 | 11·95 × 10·6 | 11·5 × 10·3 | 11·9 × 10·75 |
| 3. Diameter of pollen (in microns) | 2n | 64·5 | 59·1 | 66·4 | 62·1 | 56·2 | 66·5 | 64·2 |
| | 4n | 69·4 | 64·2 | 68·4 | 69·8 | 58·6 | 67·1 | 70·0 |
| 4. Percentage of fertile pollen | 2n | 95·1 ±0·81 | 93·2 ±0·87 | 95·1 ±0·63 | 94·9 ±0·89 | 92·2 ±0·97 | 94·2 ±0·58 | 96·9 ±0·33 |
| | 4n | 78·6 ±1·12 | 78·3 ±1·70 | 90·2 ±0·96 | 82·0 ±1·36 | 81·8 ±1·80 | 82·1 ±1·99 | 88·0 ±1·50 |

35 produced nearly equal number of pods as their diploids, and in the other three varieties they were significantly less. As the number of seeds per pod was reduced and the seed size increased in tetraploids, the pods were generally shorter but broader. But when pods having equal number of seeds were compared, those of tetraploids were invariably larger. A significant increase in size of the seed was obtained in all tetraploids. Consequently the seed weight, was about 25 per cent more in Sholapur, about 50 per cent more in Ashy Hebbal, and in the rest of the varieties the increase was between 30-37 per cent. Seed yield per plant was, however, reduced in all tetraploids, which ranged from 66 to 31 per cent of corresponding diploids.

*Meiosis.*—Meiosis was regular in the diploids. In the tetraploids at diakinesis and first metaphase quadrivalents and bivalents were very common, although univalents were by no means infrequent. Tetraploid nature of all the varieties were confirmed from meiotic studies and the frequency of the different configurations are given for three of them counted from 50 clear plates.

Anaphase disjunction, which could be clearly studied only in a limited number of cells, showed that unequal distribution of chromosomes was rather frequent. No lagging chromosomes were observed. Supernumerary spores (micronuclei) were observed in the quartets.

*Breakdown of tetraploidy.*—In the tetraploid populations ($C_2$ and $C_3$ generations) of all the varieties some plants exhibited a variety of abnormal features. The more important and commonly occurring abnormalities being total suppression of apical bud, extremely slow growth of the apical bud resulting in small-statured (miniature) plants, and plants with conspicuously thicker or thinner leaves.

TABLE 4

*Mean number of pods with* 1-7 *seeds and total pods per plant*

| No. of seeds per pod | | Dharwar | Bijapur | Sholapur | Hyderabad | Co. 35 | Ashy Hebbal | Co. 4 |
|---|---|---|---|---|---|---|---|---|
| 1 | 2n | 6·1 | 15·6 | 10·5 | 24·8 | 8·5 | 14·3 | 22·2 |
| | 4n | 10·3 | 46·4 | 46·9 | 92·6 | 64·5 | 76·8 | 87·3 |
| 2 | 2n | 16·2 | 21·3 | 13·5 | 30·7 | 13·1 | 19·0 | 33·2 |
| | 4n | 6·6 | 34·8 | 25·8 | 48·3 | 26·7 | 49·6 | 60·6 |
| 3 | 2n | 15·8 | 30·0 | 15·5 | 31·6 | 15·0 | 23·5 | 42·0 |
| | 4n | 4·5 | 22·6 | 14·0 | 18·4 | 10·0 | 26·0 | 30·5 |
| 4 | 2n | 19·6 | 41·6 | 18·3 | 28·1 | 23·0 | 29·6 | 51·2 |
| | 4n | 1·8 | 8·8 | 6·7 | 4·1 | 2·5 | 11·5 | 15·7 |
| 5 | 2n | 13·3 | 41·2 | 34·3 | 19·1 | 28·1 | 28·5 | 58·5 |
| | 4n | 0·4 | 5·0 | 2·1 | 1·6 | 1·1 | 2·3 | 4·4 |
| 6 | 2n | 3·0 | 33·2 | 16·1 | 7·5 | 22·2 | 23·2 | 39·0 |
| | 4n | .. | 1·5 | .. | 0·2 | .. | 0·5 | 1·0 |
| 7 | 2n | .. | 3·6 | 2·0 | .. | 4·1 | 3·1 | 7·0 |
| | 4n | .. | .. | .. | .. | .. | .. | .. |
| Total pods | 2n | 74·0 | 186·5 | 100·2 | 141·8 | 114·0 | 141·2 | 253·1 |
| | | ±12·2 | ±25·4 | ±14·5 | ±28·8 | ±17·4 | ±17·4 | ±39·0 |
| | 4n | 23·6 | 119·1 | 95·5 | 165·2 | 104·8 | 166·7 | 199·5 |
| | | ±8·2 | ±14·4 | ±28·0 | ±19·8 | ±19·0 | ±29·0 | ±23·3 |

TABLE 5

*Mean weight of* 100 *seeds and seed yield per plant*

| | | Dharwar | Bijapur | Sholapur | Hyderabad | Co. 35 | Ashy Hebbal | Co. 4 |
|---|---|---|---|---|---|---|---|---|
| 1. Weight of 100 seeds (gms.) | 2n | 4·00 | 3·15 | 3·11 | 3·45 | 4·05 | 3·02 | 3·25 |
| | | ±0·04 | ±0·06 | ±0·10 | ±0·04 | ±0·10 | ±0·04 | ±0·02 |
| | 4n | 5·39 | 3·95 | 4·05 | 4·60 | 5·43 | 4·67 | 4·44 |
| | | ±0·06 | ±0·06 | ±0·03 | ±0·04 | ±0·04 | ±0·05 | ±0·03 |
| 2. Seed yield per plant (gms.) | 2n | 8·38 | 19·10 | 11·67 | 16·78 | 14·33 | 18·55 | 34·31 |
| | | ±1·24 | ±2·80 | ±1·75 | ±2·76 | ±3·30 | ±2·30 | ±5·70 |
| | 4n | 2·92 | 9·15 | 5·10 | 7·80 | 4·44 | 12·34 | 11·22 |
| | | ±1·65 | ±1·25 | ±1·60 | ±0·93 | ±0·29 | ±1·80 | ±1·66 |

Pollen fertility of these odd plants was highly variable. Owing to the very small size of the chromosomes and difficulty encountered in obtaining good meiotic stages, it has been possible to etablish the nature of the chromosome complement in only a few plants, three of which were found to be aneuploids. Two of the plants belonging to the variety Ashy Hebbal had 39 chromosomes (4n-1) and one belonging to the variety Bijapur had 38 chromosomes (4n-2).

TABLE 6

*Range and mean frequency per p.m.c. of different chromosome configurations at metaphase I in the three tetraploids*

| Variety | IV | | III | | II | | I | |
|---|---|---|---|---|---|---|---|---|
| | Range | Mean | Range | Mean | Range | Mean | Range | Mean |
| Ashy Heb. | 3–9 | 5·82 | 0–8 | 1·30 | 0–12 | 5·17 | 0–6 | 2·35 |
| Hyderabad | 5–8 | 6·25 | 0–1 | 0·64 | 2–12 | 5·43 | 0–4 | 2·20 |
| Coimb. 4 | 4–9 | 6·36 | 0–11 | 0·45 | 1–10 | 5·36 | 0–4 | 2·45 |

*Aneuploid I.*—The main axis and branches were shorter and slender, and branches were fewer. The leaves were significantly smaller. The pods and seeds were smaller and narrower. Meiotic studies in pollen mother cells revealed the presence of only 39 chromosomes (4n-1) at metaphase I and anaphase I. Supernumerary spores ranging from 1 to 6 were present in nearly 80 per cent of the quartets, which was considerably greater than in normal plants.

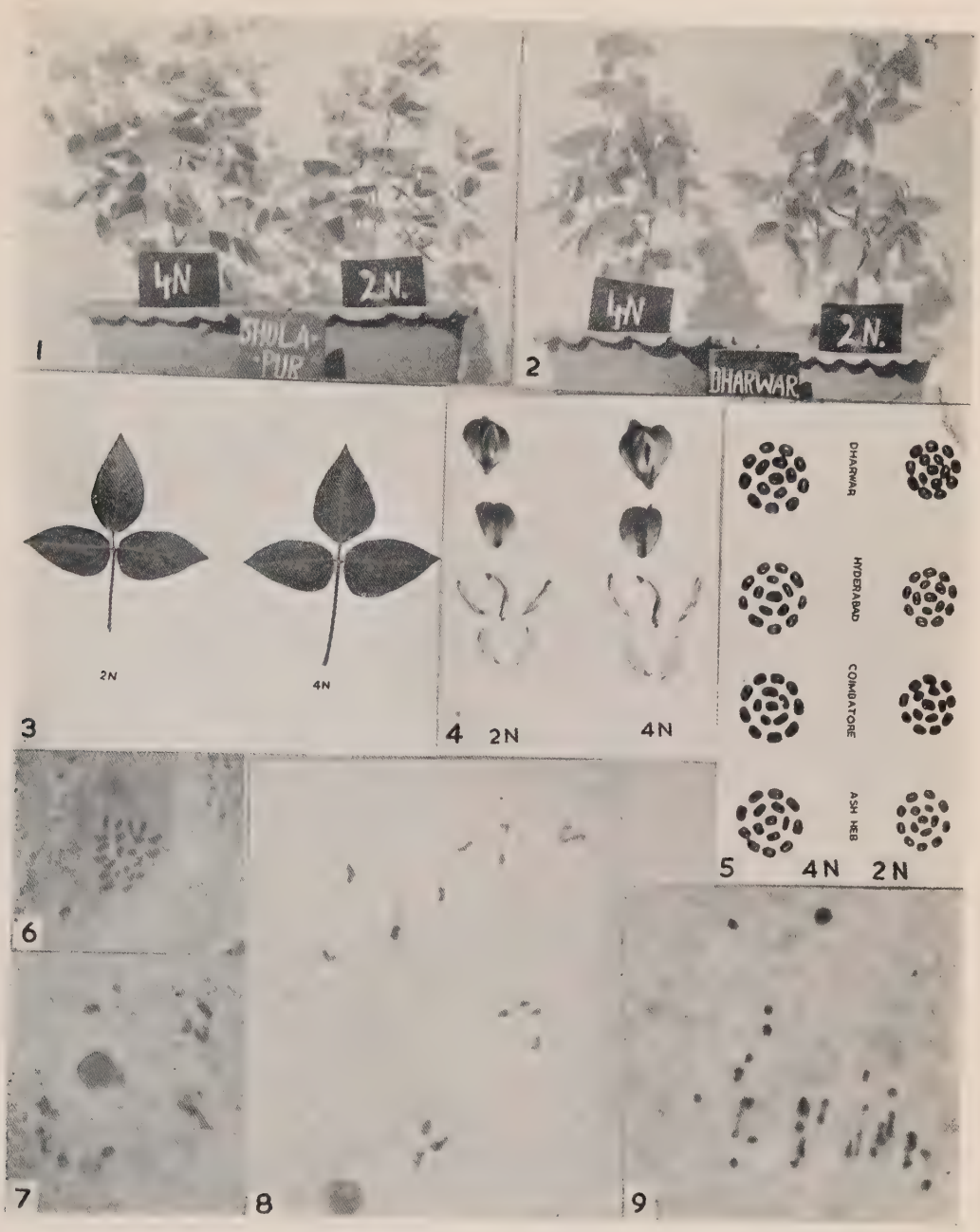
The pollen fertility of the two plants was 74·0 and 70·0. The percentage of four-germ-pored pollen in these plants was only 27·7, while in the tetraploids it was as high as 73·1.

*Aneuploid II.*—The plant was extremely slow growing, short-statured with only two branches. But the leaves were larger and thicker than normal tetraploid leaves. Flowers were larger and majority of them cleistogamous.

The plant had only 38 chromosomes (4n-2). At metaphase I fewer multivalents (trivalents and quadrivalents) were seen, but an abnormally high proportion of univalents, 7·5 per cell, has been observed. The frequency of different configurations at metaphase I in 10 cells examined was found to be 2·9-IV, 1·8-III, 6·7-II and 7·5-I. As would be expected with such high frequency of univalents, anaphase disjunction was unequal. Supernumerary spores occur in nearly 90 per cent of the quartets; the maximum number observed per quartet was nine.

## DISCUSSION

Of the two methods used for colchicine treatment, the seed treatment failed to give any colchiploid inspite of the wide range of doses tried, while the treatment of apical buds resulted in a large number of plants with polyploid sectors. In fact all the six treatments in the latter method gave some colchiploid plants; 0·25 per cent-9 hr. and 0·5 per cent-3 hr. treatments, however, were more successful. The failure of seed treatment is due to inhibition of growth in the treated seeds, which is more severe in the plumular region. Hyun (1956) is of the opinion that this inhibition of growth is probably due to deleterious effect of colchicine on the enzyme system of seed. The effect is likely to be more deleterious in large seeds which will imbibe more colchicine solution. Among other legumes also immersion of stem-tip region in colchicine solution, or mere application of colchicine to the apical bud has been found to be more successful. Thus, Kumar *et al.*, (1945) and Bhattacharjee (1956) in pigeon pea, Kumar (1945) and Ghosh (1939) in green gram, Sen and Chheda (1958) in black gram and Bhowal (1958) in Cowpea, succeeded in inducing tetraploidy rather easily by treating apical buds with colchicine, but practically failed to do so with seed treatment. However, Ramanujam and Joshi (1941), and Srivastava (1955) obtained




Figs. 1 and 2. Tetraploid and diploid plants of vars. Sholpur and Dharwar.
Figs. 3 and 4. Diploid and tetraploid leaves and floral parts of Coimbatore 4.
Fig. 5. Tetraploid and diploid seeds.
Figs. 6 and 7. Mitotic metaphase and meiotic pairing in the diploid.
Figs. 8 and 9. Diakinesis and metaphase pairing in the tetraploid.

better results in varieties of gram with seed treatment, while Bragdo (1955) reported that in red and alsike clover the seed treatment, though it had less survival, nevertheless gave higher percentage of tetraploids.

The germination of both diploid and tetraploid seeds in the field commenced at about the same time. The percentage of germination of the tetraploids is either nearly equal to the corresponding diploids or slightly reduced in the different varieties. A number of workers, however, have reported considerable delay in germination of tetraploid seeds. As regards germination capacity of tetraploids while some workers (Greis, 1948, in barley) reported considerable reduction, Müntzing (1951) in Steel rye reported an increase.

Gigantism, the usual characteristic feature of tetraploids, is seen in different plant parts. Marked varietal differences have been noticed among the tetraploids of the seven varieties in the various morphological and physiological characters.

The tetraploids are slow growing in the early stages as seen by their reduced height and lesser number of branches, but either equalled or even surpassed the diploids in height towards the concluding stages. The number of branches in tetraploids, however, are less even at the end, but both the main axis and the branches are thicker. Reduced number of branches has been commonly observed in other autotetraploid branching legumes. The increase in thickness of tetraploid stem is mainly due to increase in size of the constituent cells and to some extent due to the wider medullary cavity. Secondary structure of the stem in tetraploid is characterised by wider medullary rays and cells with thinner walls.

The leaves of tetraploids are broader and thicker. The increase in thickness is due to increase in size of the cells and all the three regions—epidermis, palisade and spongy parenchyma contributed equally to the increase.

Delayed flowering, an invariable consequence of chromosome doubling, is also seen in the present material. The difference in the commencement of flowering between diploid and tetraploid is considerably narrowed down in the late flowering varieties. The flowering period is, however, prolonged by 4-6 weeks in all tetraploids. The delayed flowering of tetraploids has been attributed, like other physiological phenomena, to slower rate of metabolic activity and their continued flowering has probably been due to less exhaustation of the plants in fruit setting, which is usually reduced. All the floral parts are larger in tetraploids and the increase is generally greater in width than in length.

The most remarkable feature of tetraploid horse gram is the production of pollen in similar abundance as diploids, a high percentage (80-90 per cent) of which is stainable, unlike what has been observed normally in autotetraploids. The size of the grains is more variable in tetraploids and the mean showed only slight increase. A characteristic feature of pollen from tetraploid is the occurrence of four-germ pored pollen which constitute the major portion. The presence of four-germ pored pollen in tetraploids alone was also observed in other legumes like green gram, black gram, cowpea, etc. On the contrary, Johnson and Sass (1945) reported that in sweet clover the pollen was triangular in tetraploid and oval in diploid.

The pods of tetraploids are shorter and the number of seeds in them is usually reduced. However, when pods having equal number of seeds both in diploid and tetraploid are compared, the latter are always longer. Inspite of greater number of pods produced per plant by tetraploids of some varieties, the seed yield is reduced due to fewer seeds per pod. The maximum yield in tetraploids is obtained in the variety Ashy Hebbal which is about 66 per cent of diploid. Nevertheless some plants in tetraploid lines exceeded the yield of diploid mean.

A significant reduction in seed setting has been commonly observed in several of the induced tetraploids so far studied. This reduced fertility of autotetraploids was attributed by Darlington (1937) and Kostoff (1939, 1940) to the formation of

multivalents at synapsis and their consequent irregular distribution. In three of the tetraploid horse gram varieties in which meiosis was studied, about 60 per cent of the chromosomes formed quadrivalents and there is indication of unequal separation of chromosomes at anaphase, but lagging chromosomes and multipolar separation have not been observed. Thus, meiotic irregularities could account for only a part of the observed sterility. Since the tetraploids produce abundant stainable pollen the reduction in seed setting may be more likely due to zygotic sterility assuming that there is equally good fertility on the female side. The diploids had twenty and tetraploids forty chromosomes in all the seven varieties, thus, confirming Sen and Vidyabhusan's (1959) observation.

Among the progenies of tetraploids of all varieties a number of abnormal-looking plants (about 5 per cent) having comparatively reduced fertility could be seen. In three of these odd plants it has been possible to establish the nature of chromosome complement, two of the plants belonging to Ashy Hebbal have 39 chromosomes (4n-1) and one of Bijapur had 38 chromosomes (4n-2). Breakdown of tetraploidy has been reported in selfed progenies of several autotetraploids such as rye (Müntzing, 1943), *Eruca sativa* (Rajan *et al.*, 1950). As unequal separation of the chromosomes could be seen in the tetraploids, viability of some of the gametes with different chromosome numbers is the likely source of the aneuploids.

## Summary

1. Treating seeds with colchicine completely failed to induce tetraploidy in horse gram but several tetraploid plants resulted in all the apical bud treatments of young seedlings. Most of the affected plants were chimaeras, from the progenies of which pure tetraploids were obtained.

2. The tetraploid seeds germinate at about the same time as diploid ones but the germination percentage was reduced slightly in some varieties.

3. The tetraploids were slow growing in the early stages, but grew faster later, and together with prolonged growth period managed to equal or even surpass the diploids in height at the end. The number of branches was reduced in the tetraploids of all but one variety. The dry weight was more in the tetraploids at the end, but the differences were not significant. The mean basal diameter of the stem was increased due to the increase in size of the component cells and wider medullary cavity.

4. A significant increase in width and thickness of the leaf was observed in tetraploids; all the three layers—epidermis, palisade and spongy parenchyma contributed to the increase in thickness.

5. Flowering in the tetraploid was delayed, the delay being less in the late-flowering varieties. The flowering period was prolonged in all varieties by about 4-6 weeks. All the floral parts were intensified and the increase was more in width than in length. Pollen from tetraploids was highly variable, slightly larger, mostly with four germ pores and a large proportion stainable.

6. Fruit setting was fairly good compared to the autotetraploids in general, and in two varieties the number of pods per plant exceeded even those of the corresponding diploids. The pods were, however, smaller owing to the decrease in number of seeds per pod. Seeds of tetraploids were larger and the weight increased significantly. But the seed yield was reduced considerably.

7. In the tetraploids, about two-thirds of the chromosomes form quadrivalents, but subsequent meiotic irregularities are not very conspicuous.

8. Among the selfed progenies of tetraploids, a number of odd-looking plants could be detected, three of which were found to be aneuploids.

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# INHERITANCE OF SPININESS OF CAPSULE IN *RICINUS COMMUNIS*

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The fruit of the castor plant is generally either spiny or spineless. In crosses between spiny × spineless types, the hybrid is less spiny than the spiny parent and is designated as partial spiny. Harland (1920), Peat (1926) and Patwardhan (1931) have earlier reported that the spiny condition of the fruit is partially dominant over spinelessness and that the two characters are controlled by a single pair of factors. A new mutant, which was less spiny, and resembled the partial spiny heterozygote but bred true for this character in the subsequent generations has been isolated by the author. The inheritance of the three types viz., spiny, spineless and less spiny condition of the fruit, is presented in this paper.

## Materials and Methods

In order to study the inheritance of spininess of the fruits, the following parents were selected and crossed in all possible combinations in 1953-54:

1. 4202G (Spiny)
2. 1514A (Spineless)
3. 4101D (Less spiny).

The study of their $F_1$, $F_2$, and $F_3$ was carried out in subsequent years in space planted rows. Kraft paper bags were used for selfing.

## Observations

*Spiny vs. Spineless.*—The cross between a spiny capsule type (4202G) and a spineless capsule type (1514A) produced an $F_1$ with spiny capsules, although the density of spines was intermediate between spiny and spineless types. The spiny condition was, thus, partially dominant over spinelessness.

In $F_2$, both spiny and spineless plants were obtained. Out of 221 plants in the $F_2$, there were 166 spiny capsuled and 55 spineless capsuled plants. The data gave a good fit to 3:1 ratio, the Chi-square being 0·0013 and P being between 0·95-0·98. It is, therefore, concluded that spiny and spineless capsule characters differ by a single factor pair.

The results were further confirmed from the $F_3$ data (Table 1).

Out of 9 progenies from $F_2$ plants with spiny capsules, 5 families segregated in $F_3$ into spiny and spineless fruited plants in the expected 3:1 ratio. These were heterozygous for the spiny character. The other 4 families bred true to the spiny character and were, therefore, homozygous for spiny fruit. The seven families from spineless $F_2$ individuals bred true to the spineless character and were, therefore, homozygous for the recessive spineless character. These findings are in conformity with those of previous workers (Harland, 1920; Peat, 1926 and Patwardhan, 1931).

*Spiny vz. less spiny.*—In the cross between spiny capsule type (4301 Garden) and less spiny capsule type (4101D) the $F_1$ was like the spiny parent. Spininess was, thus, found to be dominant over less spiny type.

The $F_2$ obtained by selfing the spiny capsuled $F_1$ plants showed segregation into both spiny and less spiny fruited plants. Out of 449 plants in $F_2$, there were 346 with spiny and 103 with less spiny capsules. The segregation in $F_2$ gave a close fit to 3:1

TABLE 1

| Family | $F_2$ character | Segregation in $F_3$ No. of plants with Spiny capsules | Spineless capsules | Ratio | Chi-square | P value |
|---|---|---|---|---|---|---|
| 1. .. .. | Spiny | 10 | 6 | | | |
| 2. .. | ,, | 3 | 1 | | | |
| 3. .. | ,, | 23 | 6 | | | |
| 4. .. | ,, | 12 | 4 | | | |
| 5. .. | ,, | 13 | 4 | | | |
| Total 5 families | ,, | 61 | 21 | 3:1 | 0·016 | 0·90 |
| Total 4 families | ,, | Bred pure | | | | |
| Total 7 families | Spineless | Bred pure | | | | |

ratio as the Chi-square value was 1·016 and P lay between 0·30-0·50. It is, therefore, concluded that the spiny and less spiny capsule characters differ by a single factor pair.

The results in $F_3$ confirmed the $F_2$ results (Table 2).

TABLE 2

| Family | $F_2$ character | Segregations in $F_3$ No. of plants with Spiny capsules | Less spiny capsules | Ratio | Chi-square |
|---|---|---|---|---|---|
| 1. .. | Spiny | 8 | 2 | | |
| 2. .. | ,, | 17 | 6 | | |
| Total 2 .. | ,, | 25 | 8 | 3:1 | 0·009 |
| Total 3 families | ,, | Bred true | | | |
| Total 11 families | Less spiny | Bred true | | | |

Out of 5 progenies grown from selfed seeds of $F_2$ plants with spiny capsules, 2 segregated in the $F_3$ into spiny and less spiny type plants in a 3:1 ratio and must have, therefore, been heterozygous for the spiny character. The other three families bred pure for the spiny character and were, therefore, homozygous for the dominant spiny character. All the 11 progenies from less spiny $F_2$ plants bred true to the less spiny character, showing thereby the recessive nature of the less spiny character. It is, therefore, concluded that the spiny and less spiny characters, in this cross, are differentiated by a single factor pair.

*Spineless vs. less spiny.*—In the cross between type 9 (spineless capsules) and type 4101D (less spiny capsules), the $F_1$ was spineless. The spineless capsule character was, therefore, found to be dominant over the less spiny type.

In the $F_2$, the plants segregated into spineless and less spiny types. Although a few fruits showed one or two spines in the capsules on the main raceme, they were included in the spineless class. This was later shown to be justified by the behaviour in $F_3$. Out of 1261 $F_2$ individuals, there were 943 spineless and 318 less spiny type plants. This gave a good fit to 3:1 ratio, the Chi-square being 0·030 with P between 0·80-0·90. The two characters—spineless and less spiny—appear, therefore, to be governed by one factor pair difference.

The findings of $F_2$ are further confirmed by the results in $F_3$ (Table 3).

TABLE 3

| No. of family in $F_3$ | $F_2$ character | Segregations in $F_3$ | | Ratio | Chi-square | P value |
|---|---|---|---|---|---|---|
| | | No. of plants with | | | | |
| | | Spineless capsules | Less spiny capsules | | | |
| 1. .. | Spineless | 15 | 5 | | | |
| 2. .. | ,, | 26 | 10 | | | |
| 3. .. | ,, | 12 | 6 | | | |
| 4. .. | ,, | 11 | 4 | | | |
| 5. .. | ,, | 6 | 1 | | | |
| 6. .. | ,, | 10 | 3 | | | |
| 7. .. | ,, | 5 | 2 | | | |
| 8. .. | ,, | 10 | 2 | | | |
| Total 8 families | ,, | 95 | 33 | 3:1 | 0·041 | 0·80–0·90 |
| Total 9 ,, | ,, | Bred pure | | | | |
| Total 9 ,, | Less spiny | Bred pure | | | | |

Out of 17 spineless $F_2$ families tested in $F_3$, 8 families segregated into spineless and less spiny types in 3:1 ratio showing their heterozygous nature. The other 9 families bred pure for spineless character and must have been homozygous for the dominant character. As was expected, all 9 less-spiny families bred pure in the $F_3$ for the less spiny character proving, thereby, the recessive nature of the less spiny character.

It is, thus, concluded that spineless fruit condition is dominant over less spiny with monogenic inheritance.

## SUMMARY

The inheritance of spininess of capsule in castor was studied. Spiny capsule character was found to be dominant over spineless and less spiny capsule characters. Spineless capsule character, however, was found to be dominant over less spiny condition of the capsule. The three characters viz., spiny, spineless and less spiny, showed monogenic inheritance and form an allelic series.

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